VOLUME 2 NUMBER 4

Per

COPYRIGHT DEPOSIT.

NUTRITION REVIEWS

THE LIBRARY OF CONGRESS SERIAL RECORD
APR 18 1944

April 1944

Natural Toxicants and Nutrition 97
Replacement of Vitamins of the B-group by Amino Acids 99
Constitution of Prothrombin 101
Glucoascorbic Acid in the Production of a Scurvy-Like Condition ... 102
Crystalline Folic Acid and Agranulocytosis 103
Hypothalamic Hyperphagia and Obesity 105
Nutritional Status of Aircraft Workers 107
The Biosynthesis of Thiamin in Man 109
The Relation of Riboflavin to Ocular Rosacea 110
Placental Transfer of Vitamin C 113

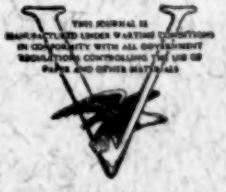
(Continued on Back Cover)

Published Monthly by

THE NUTRITION FOUNDATION, INC.

CHRYSLER BUILDING, NEW YORK, N. Y.

COPYRIGHT, 1944, NUTRITION FOUNDATION INC., NEW YORK, N. Y., U. S. A.



APR 17 1944

Editor

FREDRICK J. STARE, Ph.D., M.D., Assistant Professor of Nutrition, Schools of Medicine and Public Health, Harvard University; Associate in Medicine, Peter Bent Brigham Hospital, Boston. Editorial Office, 25 Shattuck St., Boston, Mass.

Assistant Editors

OTTO A. BESSEY, Ph.D., Division of Nutrition and Physiology, The Public Health Research Institute of the City of New York, Inc.

FLOYD S. DAFT, Ph.D., Division of Physiology, National Institute of Health, Bethesda

CARL V. MOORE, M.D., School of Medicine, Washington University; Barnes Hospital, St. Louis

WILLIAM D. ROBINSON, M.D., School of Medicine, Vanderbilt University, Nashville

ELMER H. STOTZ, Ph.D., New York Agricultural Experiment Station, Cornell University, Geneva

M. M. WINTROBE, M.D., Ph.D., School of Medicine, University of Utah, Salt Lake City

Editorial Committee

REGINALD M. ATWATER, M.D., American Public Health Assn.

GEORGE R. COWGILL, Ph.D., Yale University

CONRAD A. ELVEHJEM, Ph.D., University of Wisconsin

J. MURRAY LUCK, Ph.D., Stanford University

JAMES S. McLESTER, M.D., University of Alabama

HENRY C. SHERMAN, Ph.D., Columbia University

RUSSELL M. WILDER, M.D., University of Minnesota

JOHN B. YOUMANS, M.D., Vanderbilt University

J. C. DRUMMOND, D.Sc., University of London
CORRESPONDENT FOR ENGLAND

FREDERICK F. TISDALL, M.D., University of Toronto
CORRESPONDENT FOR CANADA

Subscriptions should be sent to the Nutrition Foundation, Inc., Chrysler Building, New York, N. Y. Rate $2.00 per year in the United States and Canada; $2.50 in other countries.

Note: **Items may be quoted from NUTRITION REVIEWS without special permission. If quotations are made, the editors ask only that credit be given to NUTRITION REVIEWS and that they be made in such a way that the original meaning of the review is preserved. Signed articles do not necessarily represent the opinion of the Editorial Staff.**

NUTRITION REVIEWS

VOL. 2 APRIL, 1944 No. 4

NATURAL TOXICANTS AND NUTRITION

In recent years, nutritional studies have emphasized particularly conditions which are associated with the lack of some essential dietary component or components, the so-called deficiency diseases. Of these, protein deficiency resulting in hypoproteinemia and nutritional edema, cobalt deficiency, and the various avitaminoses may be cited as typical.

Another kind of nutritional disorder, of which a number of examples are known, has received less attention, namely malnutrition, which is associated with natural toxicants present in food. Toxic substances may also be introduced into the food by methods of control of plant disease (use of sprays containing salts of such toxic metals as lead, arsenic, or copper) or in the processes of food technology. It is proposed to discuss a few nutritional disturbances associated with natural toxicants, not introduced, so far as known, by human activities.

Many natural toxicants are so obviously toxic that both animals and man have learned from experience to avoid them. A few plants such as fungi with toxicants of unknown nature, may be eaten by mistake. There are, however, other substances notably less toxic, the continued ingestion of which may produce chronic or subacute malnutrition. Examples of such intoxicants of both organic and inorganic nature are known.

Two natural inorganic intoxicants have received much attention in recent years. The presence of fluorine in relatively small amounts in drinking water is known to be an important factor in the defect of human teeth known as mottled enamel (Smith, Lantz, and Smith, *Ariz. Exp. Sta., Tech. Bull. no.* **32** *(1931)*). From soils derived from the geologic formation known as the Pierre shales, particularly in the Dakotas and Wyoming, selenium may be taken up in variable quantities. Cereal grains are moderate "absorbers" of selenium, but rarely contain more than 30 parts per million. On the other hand, species of certain genera (e.g., Astragalus) may accumulate several thousand parts of selenium per million. The occurrence of such seleniphilic plants in a region has been used as an indication of seleniferous soils. Since selenium compounds, in contrast to those of the closely related element sulfur, are extremely toxic, the consumption of seleniferous grains and herbage results in serious impairment of health, particularly in livestock raised in these regions. This is the "selenium" problem, which has been the subject of much concern both in animal husbandry and as a potential danger to public health (Painter, *Chem. Rev.* **28,** *179 (1941);* Moxon and Rhian, *Physiol. Rev.* **23,** *305 (1943)*).

Among the intoxicants of organic nature is gossypol, present in the cottonseed and in raw cottonseed meal. In recent industrial procedure, the seeds or meal are cooked before the cottonseed oil is expressed and the gossypol is thus inactivated or rendered nontoxic by the heat ("Accepted Foods and Their Nutritional Significance," *Am. Med. Assn., Chicago, p. 382 (1939)*). Gossypol has been shown to be an ether-soluble polyphenol of complex structure (Adams and associates, *J. Am. Chem. Soc.* **60,** *2193 (1938)*). When properly prepared, cottonseed flour may be used in any amounts which would be apt to be consumed without injurious effects, and may become important as a source of protein of vegetable origin.

Another interesting toxicant is present in the tung nut; the poisonous principle is not removed with petroleum ether, but is dissolved or destroyed by boiling with alcohol. As with cottonseed meal, steaming or simi-

lar heat treatment renders the tung meal nontoxic (Erickson and Brown, *J. Pharmacol. Exp. Therap.* **74,** *114* (*1942*)).

Many species of legumes are injurious when eaten in considerable amounts. Favism, a condition occurring particularly among the Italians of Sardinia and to some extent in the United States, is attributed to the consumption of fava beans (Vicia faba, also known as the horse bean), which are a staple article of diet and are cultivated in this country and imported as a canned food from Italy (Luisada, *Medicine* **20,** *229* (*1941*)). An amino acid, 3,4-dihydroxyphenylalanine (commonly abbreviated to "dopa") has been isolated from the seeds and pods of the fava bean, from the seeds of the Georgia Velvet Bean (Miller, *J. Biol. Chem.* **44,** *481* (*1920*)), and is presumably present in other beans of this genus (Stizolobium). "Dopa" has been isolated from the seeds of Mucuna pruriens (also of this same group), a plant widely used in India (Damodaran and Ramaswamy, *Biochem. J.* **31,** *2149* (*1937*)). While little attention has been paid to the toxicity of "dopa," it has been reported to cause nausea in man. The consumption of beans of the genus Stizolobium by man is stated to be followed by nausea and diarrhea. Whether "dopa" has any relation to favism is not known.

The djenkol bean (Pithecolobium lobatum) is relished by the natives of Java (Van Veen and Hyman, *Rec. trav. chim. Pay-Bas* **54,** *493* (*1935*)) and the Philippines (Bendaña, *private communication*), despite the fact that severe poisoning resulted from its ingestion by certain susceptible individuals. Small difficultly soluble crystals were observed in the urine of these individuals. On purification, a new sulfur-containing amino acid was obtained, to which the name, djenkolic acid, was assigned. This acid, later isolated from the beans, has been synthesized and proved to be a unique compound, the cysteine thioacetal of formaldehyde (du Vigneaud and Patterson, *J. Biol. Chem.* **114,** *533* (*1936*)). The relation of djenkolic acid to the intoxication observed after the consumption of djenkol beans is yet to be demonstrated.

Of special interest today is lathyrism, associated with the ingestion of peas of the genus Lathyrus in considerable amounts (Stockman, *J. Pharmacol. Exp. Therap.* **37,** *43* (*1929*)). Lathyrism is a common disease in central and northern India. Lathyrus peas, although used habitually as a daily article of diet by large populations in India and Algeria, have long been known to be poisonous under certain circumstances. They are harmless when eaten in moderate amounts but in periods of famine or when cereals are costly, their increased consumption has been followed frequently by epidemics of severe poisoning, especially among those who cannot afford the more highly priced cereals and pulses. Small local outbreaks have frequently occurred in Italy, France, and elsewhere in Europe. Dr. William D. Robinson (*personal communication*) observed a number of cases in Spain in the summer of 1941 and a greater incidence was reported in 1942. The disease is generally accepted to be a disturbance of the central nervous system due to an unidentified toxic principle present in certain varieties of pea, of which the chickling vetch, Lathyrus sativus, known in Spain by the name of "almortas," is most important. Experimental lathyrism has been produced in many species, including the white rat. Geiger, Steenbock, and Parsons (*J. Nutrition* **6,** *427* (*1933*)) produced lathyrism in white rats fed considerable quantities of the flowering sweet pea (Lathyrus odoratus) as did also Lewis and Esterer (*Proc. Soc. Exp. Biol. Med.* **53,** *263* (*1943*)). The toxic principle has been variously claimed to be phytin, an alkaloid, a glucoside, or an amine, but the evidence is not convincing. Sweet pea meal, previously extracted with cold water, did not cause lathyrism when fed to

rats. Since lathyrus peas are grown extensively as a forage crop, this problem is important also in animal husbandry.

It is evident that the nutrition of the peoples of other continents than our own will become a matter of increasing concern to us in the years ahead. For this reason, it is desirable to understand the nutritive value of many foods not commonly used in America. The problems of lathyrism, favism, and related conditions, and diseases of nutrition associated with intoxicants, should be investigated. It is not unlikely that other disturbances due to similar toxic factors exist and may be important, particularly in periods of food shortage or among peoples whose nutrition is at a minimal level.

HOWARD B. LEWIS,
Professor of Biological Chemistry,
Medical School, University of Michigan,
Ann Arbor, Michigan.

REPLACEMENT OF VITAMINS OF THE B-GROUP BY AMINO ACIDS

Studies of the metabolism of microorganisms have often led to results of major significance for our understanding of metabolic events in animals. An excellent example of this has been the clarification of the mechanisms of tissue metabolism made possible, to a large extent, through studies of oxidation catalysts in yeast. Likewise a number of the vitamins of the B-group were first demonstrated as growth factors for microorganisms. Two different laboratories have described an interesting relation between some of the B-complex vitamins and certain amino acids. These findings may contribute to a fuller understanding of certain deficiency diseases.

A relation between pyridoxine and the amino acid, alanine, has been described by Snell and Guirard (*Proc. Nat. Acad. Sci.* **29,** *66 (1943)*). Their initial observation was the demonstration of the existence in natural products of a substance which is derived from pyridoxine, but which far surpasses pyridoxine as a growth factor for Streptococcus lactis R. This new growth factor has been called pseudopyridoxine. Other experiments showed that the growth promoting activity of pyridoxine was strikingly increased by autoclaving it with the culture medium. This enhanced growth effect was attributed to interaction between pyridoxine and the amino acids of the medium during the heating process, and led to studies of the effects of individual amino acids in promoting the activation of pyridoxine.

These studies revealed the surprising fact that large amounts of alanine can completely replace pyridoxine as a growth factor for this organism. Maximal growth effect of S. lactis R was obtained with 3 micrograms of pyridoxine per 10 ml. of medium or with 1 to 3 mg. of alanine per 100 ml. of medium. This effect of alanine was shown not to be due to contamination with pyridoxine since both synthetic dl-alanine or alanine treated with charcoal followed by recrystallization were equally effective. Moreover, the growth promoting action of alanine disappeared after treatment with nitrous acid, whereas the pyridoxine effect was not altered by nitrous acid.

Alanine supported continued heavy growth of the organism through repeated subcultures in the complete absence of pyridoxine. The authors seem justified, therefore, in concluding that alanine in high concentrations completely replaces pyridoxine as a growth factor. It would be interesting to know whether the organisms from subcultures grown with alanine instead of pyridoxine continue to contain pyridoxine. This knowledge would throw light on the question as to whether the alanine was used for the synthesis of pyridoxine, or whether

it caused the development of a new kind of metabolism in which pyridoxine was not needed.

The amino acid glycine inhibited growth of this organism, an inhibition which was removed by additional amounts of pyridoxine or alanine, but not by other vitamins or amino acids. The inhibitory effect of glycine was clearly noted when maximum growth of the organism was permitted by addition of 1 to 3 micrograms of pyridoxine, or of 1 mg. of alanine per 10 ml. of medium. If under these conditions 2 mg. of glycine were added, growth was suppressed to the level of the control (sample without additional pyridoxine). More than 30 mg. of glycine proved toxic for this organism. Beta-alanine, dl-serine, and dl-threonine also inhibited growth, but to a lesser extent. Similarly, inhibition produced by these amino acids could be counteracted by pyridoxine or alanine.

While alanine in high concentration can replace pyridoxine, the addition of excess pyridoxine does not permit growth in the absence of alanine. S. lactis R thus appears incapable of synthesizing alanine, a surprise considering the ease with which the organism can synthesize the structurally similar lactic acid. Glycine, serine, and threonine are also among the amino acids which are essential for the growth of this organism; they become inhibitory only when added in excessive amounts.

Another strain of lactic acid bacteria, S. lactis 125, responds in exactly the same manner to additions of pyridoxine, alanine, and glycine as S. lactis R. However, the strain Lactobacillus casei is not able to substitute pyridoxine for alanine as a growth factor.

The authors discuss the possible mechanism by which pyridoxine can be completely replaced by alanine for the growth of S. lactis R. They call attention to the fact that one half of the pyridoxine ring is actually made up of alanine which, therefore, might be the precursor of pyridoxine. The inhibition of growth of S. lactis R by glycine, serine, threonine, or beta-alanine and its removal by alpha-alanine was interpreted by Snell and Guirard as due to a metabolic interference of structurally similar compounds. The existence of such a competitive interference has been demonstrated by Woods (*Brit. J. Exp. Path.* **21,** *74 (1940)*) in the inhibition of the growth factor para-aminobenzoic acid by sulfonamides. The counteraction of glycine inhibition by alanine and pyridoxine may be due to the same mechanism, since these compounds replace one another. Snell and Guirard cite a number of authors who have observed toxic effects of glycine or serine when administered to animals. It is not known whether the toxicity of these compounds for animals can be alleviated by additional amounts of pyridoxine or of alanine.

The replacement of a vitamin of the B-group by an amino acid in the nutrition of a microorganism is apparently not an isolated phenomenon. Möller (*Angew. Chemie* **56,** *199 (1943)*) reports that Streptobact. plantarum which needs thiamin in minute amounts, is able to grow in the absence of this vitamin, provided l-tyrosine is added. Although tyrosine acted only when added in a thousand times higher concentrations than thiamin, the action was not due to impurities of thiamin in the tyrosine, since synthetic tyrosine had the same effect. Moreover, the growth of Staphylococcus aureus, which is extremely sensitive to stimulation by thiamin, was not stimulated by l-tyrosine.

A most important problem for the future will be to determine whether alanine and tyrosine serve as precursors for pyridoxine and thiamin, respectively. This possibility was discussed by Snell and Guirard in regard to the replacement of pyridoxine by alanine. The other possibility mentioned before is that subcultures growing in the absence of the B-vitamins actually do not need the factor due to a change of metabolism brought about by addition of the amino acid.

CONSTITUTION OF PROTHROMBIN

Prothrombin has been considered a pseudoglobulin. Its synthesis is dependent upon an adequately functioning liver and an available supply of vitamin K. There has been no convincing evidence that vitamin K is actually a component of the prothrombin molecule, nor that calcium is a part of its structure. Significant advance, however, in our knowledge of the constitution of prothrombin has been provided by the studies of Quick (*Proc. Central Soc. Clin. Res.* **16,** *9 (1943)*). This work also promises to bring about a further elucidation of vitamin K physiology. Quick presents evidence which indicates that prothrombin is made up of two components, designated A and B, which are combined through calcium. He points out that when blood is stored, a progressive fall in prothrombin occurs. A decrease also develops when dicoumarol is administered to animals. However, when equal parts of stored plasma (prothrombin 6 per cent of normal) and of plasma from an animal which had been treated with dicoumarol (prothrombin less than 1 per cent) were mixed, the prothrombin content of the combined plasmas, as determined by the Quick method, was 100 per cent instead of the expected 3.5 per cent; complete restoration of prothrombin activity had occurred. The loss of prothrombin in the two plasmas, therefore, was not identical. One factor (component A) was lost by storage and the other (component B) by the action of dicoumarol. It was also demonstrated that aluminum hydroxide would adsorb component B, but not component A, from oxalated plasma.

Experimental evidence indicated that the two factors were combined through calcium. Use was made of goose and chicken blood because, when specimens are collected with care to avoid contamination with tissue juice, they remain fluid even though not decalcified with oxalate or citrate. Such blood, when stored, did not lose its prothrombin activity. However, when goose or chicken plasma was decalcified, the prothrombin activity fell to 10 per cent of normal in seven days. Quick concluded that as long as goose or chicken blood retained its calcium, component A remained in the prothrombin complex and was not destroyed; removal of the calcium dissociated the complex so that the lability of component A became manifest. Furthermore, while aluminum hydroxide adsorbed component B from oxalated rabbit plasma, it was unable to do so from heparinized (not decalcified) plasma. The effect of oxalate was visualized in this manner: component A-calcium-component B plus sodium oxalate gives free component A plus calcium oxalate plus free component B. The reaction is apparently reversible because resynthesis of prothrombin occurs when oxalated plasma is recalcified.

Preliminary observations indicate that inadequate synthesis of component B is responsible for the hypoprothrombinemia which accompanies vitamin K deficiency in human subjects. In a few patients with jaundice and defective intestinal absorption of vitamin K, there was a moderate decrease in component B while component A remained normal. Animals with an induced chloroform hepatitis had a reduction of both components. Without presenting the evidence, Quick also stated that component A is apparently not a part of the thrombin molecule (formed by the action of tissue juice or thromboplastin on prothrombin).

If these results can be substantiated, their implications are numerous. They indicate that vitamin K is concerned with the synthesis of only one of the principal components of the prothrombin molecule, and that calcium is contained in prothrombin. It is at least possible that specific *in vivo* deficiencies of component A may occur. They may clarify considerably the chemistry of blood coagulation. Lastly, these results

suggest that stored plasma, even though it apparently contains no prothrombin, is lacking only in component A and may be used in the treatment of hypoprothrombinemia caused by dicoumarol administration or vitamin K deficiency. Therapeutic appraisal of this last suggestion should not take long.

GLUCOASCORBIC ACID IN THE PRODUCTION OF A SCURVY-LIKE CONDITION

The majority of animals do not develop scurvy, presumably because they are able to synthesize ascorbic acid. Mice and cotton rats are two species which thrive on a diet devoid of ascorbic acid. A report by Woolley and Krampitz (*J. Exp. Med.* **78,** *333 (1943)*) describes a condition in these animals similar to scurvy which was produced by feeding a compound structurally related to ascorbic acid. Their studies open up new problems in relation to the metabolism of ascorbic acid which will no doubt be followed by a series of productive investigations.

In a course of experiments concerned with a theory of chemotherapy, Woolley and Krampitz observed that feeding of glucoascorbic acid (2,3-enediol-d-glucoheptono-1, 4-lactone) caused a condition in mice with symptoms similar to those characteristic of scurvy as seen in species susceptible to that disease. Mice were fed a highly purified ration consisting of sucrose, vitamin-free casein, fortified corn oil enriched with fat soluble vitamins, a mineral mixture, and the crystalline vitamins thiamin, riboflavin, nicotinic acid, pyridoxine, choline, inositol, and calcium pantothenate. Such a diet is adequate for good growth of mice. When 10 per cent glucoascorbic acid was added to the above ration, a severe condition was produced in which a definite sequence of events occurred.

The first symptom, occurring four or five days after the start of the experiment, was severe diarrhea, the feces becoming soft and then liquid; at the same time the normal black color changed to a golden brown. The anal region became red and swollen. At about the seventh day extensive subcutaneous hemorrhage appeared, first on the chest, and then on the legs, tail, and sides, and in the gingiva, especially below the lower incisors. These areas were fiery red in appearance. At autopsy, after the second week, the lungs and the joints of the legs and ribs were likewise fiery red. By this time the "wrists" and "knees" had become swollen and the legs were edematous. The animals were small, extremely untidy, and sick looking. Roentgen examination showed that the bones were poorly calcified as compared to those of the control animals. Occasionally alopecia appeared on the head and down the middle of the back. It is noteworthy that the teeth did not become loosened. Death occurred in all of the animals.

When only 5 per cent of the compound was fed with the diet, 37 out of 49 animals survived for three weeks and their growth was less impaired than that of animals fed 10 per cent glucoascorbic acid. When only 2 per cent of the compound was added to the diet, no signs of disease developed except slight impairment of growth. These changes were seen in adult animals as well as in weanlings, but more time was required to cause obvious changes in adults. A similar condition was also produced in cotton rats.

A curious observation was that those mice which survived when only 5 per cent glucoascorbic acid was added to the diet eventually began to gain weight, and the changes produced by the disease slowly regressed. When the level of glucoascorbic acid in the ration was raised to 10 per cent, the spontaneous cure ceased and the disease developed again. The condition was rapidly

cured by omission of glucoascorbic acid from the ration.

Studies so far suggest that the action of glucoascorbic acid is rather specific; 5 per cent of sodium araboascorbate in the diet did not produce any of the characteristic changes.

Of particular interest was the fact that when 10 per cent glucoascorbic acid was added to a natural ration consisting of yellow corn meal, milk powder, casein, linseed oil meal, alfalfa leaf meal, and minerals (Steenbock's stock ration), no signs of the condition were observed within three weeks other than a slightly diminished rate of growth. The failure of glucoascorbic acid to produce the disease when fed with a natural ration suggests that the latter may contain a substance which antagonizes the action of glucoascorbic acid. The spontaneous cure of the condition in mice suggests the establishment of increased synthetic power for such an antagonistic substance or some other detoxifying mechanism. The first supposition would be that the "antagonistic" or neutralizing material might be ascorbic acid itself. However, mice that were fed the basal purified ration plus 5 per cent glucoascorbic acid and 5 per cent ascorbic acid, developed the characteristic changes as soon as the controls which received only 5 per cent glucoascorbic acid. Further evidence against this supposition was found in experiments in which chloretone was administered. This substance is known to stimulate the synthesis of ascorbic acid, but its administration did not prevent or cure the scurvy-like condition produced by glucoascorbic acid. The plant products, cerophyl (a specially dehydrated fresh young grass) and fresh cabbage, did cure the disorder. Whether the curative power of these products might be related to their high antiscorbutic potency remains undisclosed.

The observations of Woolley and Krampitz do not bear quite the same clearcut relation to ascorbic acid deficiency as previous observations (Woolley and White, *J. Biol. Chem.* **149,** *285 (1943); Nutrition Reviews* **2,** *59 (1944)*) on thiamin deficiency caused by pyrithiamin. There remains the question of how close is the relationship of the disorder to that caused by a deficiency of ascorbic acid. The failure to find a loosening of the teeth is most perplexing, since this is an early and constant finding in scorbutic guinea pigs and represents a pathology based on a fundamental function of ascorbic acid. The hemorrhagic conditions noted have in fact led the original investigators to test the possibility that vitamin K might be concerned. This vitamin was ineffective however in preventing or curing the symptoms due to glucoascorbic acid feeding.

No direct evidence was found to indicate that the supposed interference with ascorbic acid metabolism might be due to a competition of the structurally similar glucoascorbic acid with the natural vitamin. In previous studies with pyrithiamin and thiamin deficiency, such an antagonistic action was evident. It seems likely nevertheless that the glucoascorbic acid blocks in some manner the function of the natural vitamin, and that the use of glucoascorbic acid as a specific inhibitor should in the future reveal many details of the mechanism of ascorbic acid metabolism.

CRYSTALLINE FOLIC ACID AND AGRANULOCYTOSIS

A number of recent reports have demonstrated an alteration in the vitamin requirements of the white rat produced by feeding the highly insoluble sulfonamides, sulfaguanidine, or succinylsulfathiazole (sulfasuxidine). When any of these drugs is added to purified diets containing all of the hitherto known growth requirements for the rat, a number of signs of deficiency appear. The rats fail to grow, they develop

a leukopenia and granulocytopenia, an anemia, and in some cases an increased prothrombin time. In addition, part of the hair may fall out or lose its color and panophthalmitis may occur. These changes appear to be the result of an alteration in the bacterial flora of the gastrointestinal tract whereby the coliform bacteria which are good "vitamin synthesizers" are greatly reduced in number without much change in the number of "lactobacilli streptococci, and anaerobic bacteria (vitamin requirers)" (Welch and Wright, *J. Nutrition* **25,** *555 (1943)*).

The effect of this change in the intestinal flora is to create in the rat a requirement for an external source of (a) biotin, (b) vitamin K, and (c) some component of a preparation containing folic acid (Nielsen and Elvehjem, *J. Biol. Chem.* **145,** *713 (1942)*); Daft, Ashburn, and Sebrell, *Science* **96,** *321 (1942)*; and Black, Overman, Elvehjem, and Link, *J. Biol. Chem.* **145,** *137 (1942)*).

It has now been shown that crystalline folic acid will overcome the leukopenia and granulocytopenia, and to some extent at least, the anemia resulting from treatment with insoluble sulfonamides (Daft and Sebrell, *Pub. Health Reports* **58,** *1542 (1943)*).

Weanling white rats were placed on a purified diet containing 1 per cent sulfaguanidine or sulfasuxidine. The diet was supplemented with thiamin, pyridoxine, riboflavin, calcium pantothenate, nicotinic acid, choline chloride, cod liver oil and, toward the end of the experiment, crystalline biotin.

After a period ranging from four to fifteen weeks on this diet, the total leucocyte count was greatly reduced. Rats were then chosen which had not more than 200 granulocytes per mm^3 and these were given crystalline folic acid by mouth. The response observed was dramatic. For example, when 20 micrograms of folic acid were given daily for four days the granulocytes rose from an average of 30 per mm^3 to 5600 per mm^3, and the other leucocytes increased from 2670 to 8800 per mm^3.

Folic acid produced a marked gain in weight and had a definite but less striking effect on the anemia which many of the rats developed on the sulfonamide-containing diet. Nine rats with hematocrits of less than 30 volume per cent were given crystalline folic acid. Seven of the rats showed improvement at the end of ten days. At this time the average hematocrit had increased from 29.8 to 42.1 per cent and the hemoglobin had risen from 9.7 to 12.8 g. per cent.

Xanthopterin, which may be a component of folic acid, and which rat liver can apparently convert *in vitro* to folic acid (Wright and Welch, *Science* **98,** *179 (1943)*), was completely ineffective in raising the leucocyte count, or in producing any weight gain.

From this report it would seem clear that a folic acid deficiency develops as a result of the feeding of insoluble sulfonamides. There remains a slight possibility that folic acid is required by the remaining intestinal bacteria which in turn might synthesize accessory substances other than folic acid necessary for the rat. If this were true, at least some of the effects of folic acid would be indirect. This is not entirely unlikely, since apparently a prolonged prothrombin time—presumptive evidence of vitamin K deficiency—develops in sulfaguanidine-treated rats only if biotin and folic acid are missing from the diet. This seems to indicate that biotin and folic acid are necessary for the growth of certain intestinal organisms which in turn produce vitamin K for the rat. Now that crystalline folic acid is available, it should be possible to decide whether its effect is direct or indirect, by injecting rather than feeding the vitamin.

It is obvious that the observation that folic acid is required for the formation of leucocytes is of great importance for future

studies. It remains to be seen whether folic acid will prevent the leukopenia which is occasionally observed clinically with the soluble sulfonamides or the agranulocytosis resulting from the use of therapeutic agents such as pyramidon.

HYPOTHALAMIC HYPERPHAGIA AND OBESITY

It seems almost fantastic that albino rats could gain 20 to 23 g. in weight within eighteen hours or 100 g. in a week to ten days. Yet gains of that magnitude have been observed by Brobeck, Tepperman, and Long (*Am. J. Physiol.* **133,** *468 (1941)*; *Yale J. Biol. Med.* **15,** *831 (1943)*) in rats with hyperphagia following experimentally produced damage to the hypothalamus.

The first description of hypothalamic obesity appeared in the clinical literature over one hundred years ago (Mohr, *Wchnschr. f. d. ges. Heilk. 565 (1840)*). It was not until sixty years later, however, that theories as to the etiology of the condition were advanced. Fröhlich (*Wien. klin. Rundschau* **15,** *883, 906 (1901)*) believed the obesity to result from pituitary damage which failed to produce acromegaly. Erdheim (*Sitzungsb. d. k. Akad. d. Wissensch. Wien.* **113,** *537 (1904) Abt. III*) on the other hand pointed out the relative lack of damage to the hypophysis in some reported cases and concluded that a lesion of the base of the brain produced the condition. He was unable, however, to identify the structure concerned. The subject was considered controversial until Smith (*J. Am. Med. Assn.* **88,** *158 (1927)*; *Am. J. Anat.* **45,** *205 (1930)*) showed that while obesity in rats followed injury involving both the hypophysis and the hypothalamus, none resulted when the damage was restricted to the hypophysis alone. Hetherington (*Endocrinology* **26,** *264 (1940)*) and Hetherington and Ranson (*Anat. Rec.* **78,** *149 (1940)*) were the first to succeed in producing hypothalamic damage without hypophyseal involvement. Obesity resulted. Hetherington and Ranson (*Endocrinology* **31,** *30 (1942)*) also succeeded in producing typical hypothalamic obesity in hypophysectomized rats. The conclusion appears sound, therefore, that this type of obesity results from damage to the hypothalamus, and that any concomitant hypophyseal injury is etiologically important only in so far as it is a cause of hypothalamic pathology.

Although this condition has been studied extensively for thirty years, no previous attempt appears to have been made to discover the relationship between the amount of food consumed and the deposition of fat. Many clinicians, it is true, believe overeating to be the direct cause of hypothalamic as well as other types of obesity. The researches of Brobeck and associates are the first, nevertheless, to give convincing experimental support to this clinical hypothesis.

Lesions of the hypothalamus were produced in 200 to 300 g. albino rats by means of an electric current. Most of these animals began to eat ravenously as soon as food was offered. Of a small number given access to food before they had completely recovered from the effects of the anesthesia, one suffered fatal dyspnea from inhalation of food particles. This rat had distended its stomach esophagus and pharynx with food. Because of their voracity the rats increased in weight by as much as 20 to 23 g. within eighteen hours following operation, most of the gain representing food and water in the gut. This behavior did not persist but the measured food intake was frequently two to three times that of the controls for periods as long as twenty-five days.

There was a definite correlation between the level of food intake and the rate of weight gain. During short periods of intense

hyperphagia, weight gains were recorded of as much as 15 g. per day in excess of that of the control animals. Excess gains of 5 to 10 g. per day for relatively long periods of time were not uncommon.

It should be noted in this connection that Hetherington and Ranson (*Am. J. Physiol.* **136,** *609 (1942)*) reported that only 4 of 6 rats with hypothalamic obesity had a greater food intake than litter mate controls and stressed the importance of an observed decrease in activity of the rats with lesions. The reason for the discrepancies between these results and those of Brobeck *et al.* is not clear. The latter authors do not report a decrease in activity of their rats with hypothalamic injury and remark on several differences in experimental conditions.

Paired feeding experiments of Brobeck and associates support the general thesis that the rate of weight gain is quantitatively related to the excess of food ingested. In 9 of 12 pairs, the rats with lesions gained at the same rate as the controls, only to become obese later when they were allowed larger amounts of food.

As is frequently the case, the exceptions to the general rule are of particular interest. In 3 of 12 pairs of animals, the rats with lesions gained more rapidly than the controls receiving the same amount of food. It was noted that the greatest discrepancy was in an animal which ate its daily portion of food in the shortest time and it was thought possible, therefore, that the phenomenon might be related to feeding habits. Observations which appear to support this hypothesis were made by Tepperman, Brobeck, and Long (*Yale J. Biol. Med.* **15,** *855 (1943)*). Although high respiratory quotients were found in rats with lesions, the respiratory quotients of control rats trained to eat their daily allotment of food in three hours or less, were equally high. The authors conclude that periodic overnourishment (in trained rats) or chronic overnourishment (in rats with hypothalamic hyperphagia) results in an increased tendency to convert carbohydrate to fat. Unfortunately no data are presented concerning the weight changes of the trained untreated animals in comparison with untrained pair-fed controls. It is not entirely clear, therefore, that the excess weight gains of the 3 rats with hypothalamic lesions in the paired-feeding experiment can be satisfactorily explained on the basis of altered feeding habits alone.

A combination of hypothalamic injury and the pancreatic protein-containing diet of White and Sayers (*Proc. Soc. Exp. Biol. Med.* **51,** *270 (1942)*) produced the heaviest rats of which we have record. Two of the male rats attained weights of 982 and 962 g. respectively, while control uninjured rats fed the same pancreatic protein-containing diet weighed from 600 to 700 g. The animals with lesions given other diets were all females. The heaviest of these weighed 850 g.

Brobeck, Tepperman, and Long (*Yale J. Biol. Med.* **15,** *893 (1943)*) investigated the effect of experimental obesity upon carbohydrate metabolism, particularly in relation to the production of diabetes. Spontaneous glycosuria with impaired glucose tolerance was observed in one obese rat with hypothalamic hyperphagia. In a number of partially pancreatectomized rats without glycosuria, injury to the hypothalamus caused a large increase in food consumption and glycosuria as great as 6 to 8 g. per day. Similar animals with food intake restricted to the preoperative level did not become diabetic. The clinical significance of these observations is discussed.

This series of papers constitutes an important contribution to our knowledge of the etiology and the physiology of obesity. Direct experimental support of the concept that overeating is the direct cause of obesity was badly needed. The establishment of a causal relationship between abnormal feed-

ing habits and an increased tendency to convert carbohydrate to fat represents a significant advance of knowledge and the observations concerning excessive food intake and pancreatic diabetes should lead to further investigations of this relationship.

NUTRITIONAL STATUS OF AIRCRAFT WORKERS

The role of adequate nutrition in maintaining the health of the worker as the basic resource in our productive capacity has been deservedly emphasized. In an effort to guarantee an optimum dietary intake, measures have been taken frequently without an attempt to determine the state of nutrition of the workers or to ascertain what has been gained by the changes. The Committee on Nutrition in Industry has recognized the need to "determine the facts concerning the influence of diet and nutrition on health, working capacity, incidence of accidents, absenteeism, and the psychological state" (*National Research Council Reprint & Circular Series* no. **110,** (*1942*)). The study of Borsook, Alpert, and Keighley (*Milbank Memorial Fund Quart.* **21,** *115* (*1943*)) in an aircraft factory in Burbank, California, is of interest not only because it presents the findings in a study of 1173 male workers which will serve as a base line against which changes after known dietary supplementation can be measured, but also because the discussion of choice of methods used and the interpretation of results provides a valuable commentary on the tools which are available for the appraisal of nutritional status.

The subjects were presumably in good health, having passed a preemployment examination. Seventy-three per cent were under 30 years of age; 69 per cent had resided in California for a year or more, and the great majority of the remainder came from western states. The dietary records of these subjects have been reported by Wiehl (*Milbank Memorial Fund Quart.* **20,** *329* (*1942*)) and reviewed in this journal (*Nutrition Reviews* **1,** *238* (*1943*)). The major inadequacies of the diets appeared to be in riboflavin, calcium, and ascorbic acid, although only 2 per cent of the diets contained satisfactory amounts of all of five protective food groups.

Borsook and associates found the height-weight relationship to be in accord with standard height-weight tables, with 20.8 per cent weighing 10 per cent or more above the standard and 18.1 per cent weighing 10 per cent or more below the standard.

In the search for evidences of vitamin A deficiency, chief reliance was placed on the changes in opacity and thickness of the bulbar conjunctiva detected by biomicroscopy. Tests of dark adaptation were not done because of the difficulties in interpreting results of a single determination in terms of vitamin A nutrition, the probability that abnormalities reflect only recent nutrition, and limitations of time and personnel. The last two factors also mitigated against the use of blood vitamin A measurements. The authors raise the question as to what degree of follicular hyperkeratosis can be considered indicative of vitamin A deficiency and point out the lack of specificity of this finding. Extensive follicular hyperkeratosis was present in 1.5 per cent of their subjects. The examination of the bulbar conjunctiva revealed one or more elevated areas visible on gross examination in 50 per cent of the entire group, with an additional 32 per cent with spots seen as elevated only through the biomicroscope. In only 2 per cent was the major portion of the conjunctiva sufficiently translucent for the network of fine scleral vessels to be seen.

The only evidences of thiamin deficiency detected were mild changes in the neurologic

examination, present in 17 per cent of the subjects, which are admittedly not pathognomonic. Plantar dysthesia was by far the most frequent finding. About 26 per cent of the subjects showed loss of vibratory sensation in toes to a C-256 tuning fork, which is stated to be pathologic in men under 35 years of age. Limitations of time and personnel excluded the use of laboratory tests for thiamin deficiency.

Evidences of riboflavin deficiency were grouped into three categories: changes in skin of face and oral lesions regarded as evidence of acute deficiency, certain ocular symptoms, and the presence of capillary invasion of the cornea as seen with the biomicroscope. In the first group, 9 per cent of the subjects presented lesions, the majority being sebaceous plugs and seborrhea of the face. Thirty-five per cent of all subjects complained of burning or itching of the eyes, 25 per cent of eye strain, and 43 per cent of photophobia. All subjects showed some degree of vascularization of the cornea, and in 42.5 per cent this extended a considerable distance into the cornea. In view of the high incidence of ocular complaints and findings it should be noted that nearly all of these subjects were from the "swing shift" and worked part of their time under artificial illumination. The authors cite some of the observations which question the specificity of corneal invasion as an evidence of riboflavin deficiency and recognize that it would be premature to consider the presence of corneal vascularity in every subject examined as due to riboflavin deficiency.

Niacin deficiency, as judged by definite changes in the tongue and skin, was detected in 2 per cent of the subjects.

No evidence of scurvy was found on physical examination, but the plasma ascorbic acid was below 0.4 mg. per 100 ml. in 32.5 per cent of the subjects, and below 0.2 mg. in 11 per cent. In interpreting these figures the authors appose the studies indicating that the vitamin C depot is nearing depletion when the plasma concentration falls below 0.45 to 0.5 mg. per 100 ml. to the experience of Crandon, Lund, and Dill (*New Engl. J. Med.* **223,** *353 (1940)*) who in experimental scurvy found the plasma level to fall to zero seven weeks before the first clinical manifestation developed. From the data in the latter experiment, they conclude that from the viewpoint of prophylaxis, the conservative position would be that a plasma ascorbic acid below 0.2 mg. per cent is unsafe. While recognizing that plasma levels reflect only recent dietary intake it is believed probable that this determination in a large group of individuals does reflect their characteristic and habitual diets.

Serum albumin was determined in 195 out of 200 consecutive subjects. None fell below 3.95 g. per 100 ml., and this determination was discontinued.

Hemoglobin and packed cell volume were determined on all subjects; in addition, erythrocyte counts were done on 228 subjects selected at random, and on 110 subjects who had hemoglobin levels below 13.4 g. per 100 ml. The interpretation of the hematologic data was complicated by the tendency of the hemoglobin values to be lower than Wintrobe's normal range and for the red blood cell counts and packed cell volumes to be slightly higher. Hemoglobin values below 13.4 g. per 100 ml. were found in 10.8 per cent of the subjects, which was considered a conservative lower limit of hemoglobin for adult men. In this "anemic" group the subjects had fewer red cells and a lower color index than did the unselected series of 228 men. It should be noted that most of these "anemias" were mild, the hemoglobin falling below 12.5 g. in only 1 per cent of all the subjects.

Certain symptoms from the medical histories, while admittedly not pathognomonic of any deficiency, were noted to improve after administration of various dietary supplements. Of interest was the finding that 12 per cent found their work excessively tiring, and that 40 per cent had some type of gastrointestinal complaint, constipation

and indigestion being most frequently encountered. The authors consider the high incidence of ocular and gastrointestinal complaints and the presence of anemia in their group to be especially significant because their subjects represent a selected group of young men whose health, as evidenced in the preemployment examination, had been a real and important item in selection for employment.

This study illustrates that the apparent incidence of a specific nutritional deficiency in any group depends more upon the criteria used for detecting that deficiency than upon any other factor. It also illustrates the variation in sensitivity of the methods at present available for detecting such deficiencies. Use was made of the test of vitamin C nutrition which is capable of detecting early and readily reversible degrees of subsaturation, but with respect to niacin reliance had to be placed on anatomic changes of the acute classic deficiency disease. It would seem important in the interpretation of a test of nutritional status to have clearly defined the stage in the development of a deficiency which it is capable of detecting, whether it be at the level of subsaturation, disturbance of function, or anatomic changes of an acute or chronic nature.

The need for an accurate evaluation of the tests available for detecting mild degrees of deficiency states is self evident. Studies such as this, in which it is possible by reexamination to observe the effect of nutritional supplementation for a year or more on several hundred subjects, can be expected not only to clarify the validity and usefulness of these diagnostic tests, but also to yield factual information regarding the influence of nutrition on health and productive efficiency.

THE BIOSYNTHESIS OF THIAMIN IN MAN

The deficiency symptoms in rats produced by administration of difficultly absorbed sulfonamides make it appear likely that various members of the vitamin B-complex may be synthesized by the intestinal flora and made available for the needs of the animal. Specifically in thiamin, although it is possible that this vitamin is synthesized in the rumen of certain ruminant animals, no evidence has hitherto been offered to indicate biosynthesis in man.

Najjar and Holt (*J. Am. Med. Assn.* **123,** *683 (1943)*) fed a diet low in thiamin to 9 young men from 16 to 23 years of age, who were living a sedentary life in an institution. This diet consisted of vitamin free casein, Crisco, dextri-maltose, a mineral mixture, and a mixture of crystalline vitamins. These included ascorbic acid, riboflavin, niacinamide, calcium pantothenate, pyridoxine, choline chloride, inositol, and para-aminobenzoic acid, presumably in adequate amounts, as well as thiamin in varied amounts. Cod liver oil was also given. The diet was furnished in quantities approximating 40 calories per kilogram. This monotonous regimen was continued for eighteen months during which time weight and vigor were maintained.

The purpose of the experiment was to determine the minimum thiamin intake that would prevent thiamin deficiency. From an initial level of 1 mg. the thiamin content of the diet was gradually reduced. When the intake was as low as 0.4 to 0.6 mg. per day, the thiamin excretion in the urine fell to negligible figures as measured by the ordinary thiochrome procedure. By a more sensitive modification of this test it was found that 15 to 25 micrograms a day were excreted in the urine. No ill effects were observed in the subjects, nor were changes noted even when the thiamin intake had been as low as 0.1 and 0.2 mg. per day

for months. No significant changes were observed in electrocardiograms or in roentgen observations on the motility of the gastrointestinal tract.

Because of the lack of all signs of thiamin deficiency, thiamin was omitted altogether from the diet. In the course of the next three to five weeks, 4 of the 9 subjects developed "neuritis" and edema as well as loss of appetite and sometimes vomiting. Only these last two symptoms developed in one subject, while the remaining 4 showed no signs of deficiency during a seven week period of observation.

Repeated examinations were made of the diet, but these failed to show any trace of thiamin. The stools were also examined and then it was found that the 4 subjects who had developed deficiencies had almost no free thiamin in their stools. On the other hand large quantities of free thiamin were found in the feces of the subjects who remained free of symptoms. The content of "combined thiamin" (thiamin liberated by treatment of the feces with clarase) in the stools was very irregular.

The possibility that the thiamin content of the feces might have represented thiamin excreted into the intestine was explored by administering 50 mg. of thiamin intravenously to one subject daily for one week and measuring the thiamin content of the stools. A negative result was obtained.

Since it seemed implausible that the stores of thiamin in the body had not been completely exhausted, it appeared possible that the thiamin found in the stools of the subjects who failed to develop deficiency was derived from intestinal bacteria. This possibility was supported by the fact that in one subject the fecal content of free thiamin was reduced in a week from 27 to 52 micrograms per day to zero following the administration of 1.5 g. succinylsulfathiazole every four hours for one week. The fact that retention enemas containing 50 mg. of thiamin given to 2 persons showed a pronounced rise in urinary thiamin suggested that thiamin can be absorbed from the large intestine.

These investigations are of outstanding significance among recent contributions to human thiamin nutrition. A considerable biosynthesis of thiamin, particularly if variable, may account for some of the disagreement prevalent in determining the requirement for man, as well as for the frequently recorded disagreement between chemical and animal assay of thiamin.

It is striking, in view of reports of others on the levels of thiamin required to prevent symptoms of subclinical thiamin deficiency, to note the very low levels of thiamin intakes that were maintained for long periods of time. It might be implied from the report that only traces of dietary thiamin are required to prevent symptoms of deficiency, or simply that the specific dietary regimen used was unusually conducive to bacterial synthesis in the intestinal tract. Whether differences in the experimental and natural conditions can be held accountable, it cannot be refuted that clinical and physiologic signs of thiamin deficiency occur frequently in individuals where it is unlikely that the dietary thiamin restriction is as severe as recorded in these experiments.

THE RELATION OF RIBOFLAVIN TO OCULAR ROSACEA

The condition rosacea has been known since the Middle Ages. At first it was confused with common acne and was called acne rosacea, but later the two entities were separated, particularly as the result of observations of the Viennese dermatologist, Unna. Rosacea affects the skin and the eyes and it is readily recognized when both these structures are affected. Some clinicians do not consider that the entity of

ocular rosacea exists unless the face is also affected, but others are willing to make the diagnosis of ocular rosacea even in the absence of facial lesions. In the eye, rosacea affects the lids, the conjunctiva, and the cornea. The affected lids are characterized by red and slightly thickened margins and frequently an oily secretion or small crusts and scales are seen near the roots of the cilia. When the skin of the lids is stretched, a superficial vascular network is usually prominent. Rosacea conjunctivitis is characterized by vascular dilatations in all cases, and conjunctival nodules in some. Corneal involvement varies from a symptomless, mild, superficial vascularization of the cornea at the limbus to a severe superficial and deeper vascularization of the cornea attended by infiltrates and ulcerated areas.

Ocular symptoms may be entirely absent or they may consist of a feeling of "heaviness" about the eyes, dryness, or burning. In the cases of conjunctivitis there is frequently a thin watery or slightly mucoid discharge and the patients are apt to complain of their red eyes. When the cornea is involved there may be unusual sensitiveness to light, spasm of the lids, copious tearing, and pain. The onset of ocular rosacea is insidious, the condition is chronic, and the patient is subject to spontaneous remissions and exacerbations.

The etiologic factors which have been suggested are numerous and include all the fads which have from time to time attracted attention. They include focal infection, gastrointestinal disturbance, endocrine imbalance, lowered gastric acidity, and more recently vitamin deficiency. The last theory is based on the well known observations of Bessey and Wolbach (*J. Exp. Med.* **69,** *1 (1939)*) on the development of corneal vascularization in rats placed on a riboflavin deficient diet. Since riboflavin plays so important a role in tissue respiration, it has been suggested that it is important in relation to an avascular tissue such as the cornea.

Wise (*Am. J. Ophth.* **26,** *591 (1943)*) has made a painstaking study of ocular rosacea and has presented observations on 60 cases. Half of this number were cases of facial and ocular rosacea, while in the other half only the face was affected. The patients' age varied between 21 and 71 years; there were more women than men in the series. The study includes a careful analysis of the mode of onset, the presence or absence of digestive disturbances, and the possible relationship of menstruation and pregnancy as well as of emotional factors. The eye complications followed the facial disturbances by an interval varying from a few months to as long as thirty years. None of the factors mentioned above seemed to have any distinct relationship to the symptoms. Most patients agreed that exposure to wind, cold, or sun, eating too much or too rapidly, or drinking hot beverages or alcoholic drinks, usually made them temporarily worse. In a number of cases, there seemed to be an unusual tendency to flush. Focal infection did not seem to have any relationship to the disease, although local infections, such as sties, were frequent complications. Temporary relief had been observed by these patients when almost any new form of treatment was given.

The patients were examined particularly for signs of riboflavin deficiency. Two showed cheilosis, 3 gave a questionable history of fissures at the angles of the mouth, 5 showed mild nasolabial seborrhea, and one or 2 patients had scattered comedones about the forehead and cheeks. None of the patients showed the glossitis stated to be a part of the syndrome of riboflavin deficiency.

Of the various studies made on these patients the most noteworthy is the finding that cultures from the conjunctiva were positive for Staphylococcus in 60 per cent of 38 cases of facial and ocular rosacea, and in 23 per cent of 22 cases of facial rosacea, whereas such cultures were positive in only 4.5 per cent of 200 controls.

Riboflavin therapy was given in 21 cases.

The vitamin was given by mouth at first but as results failed to appear it was given intravenously in doses as high as 20 mg. Such treatment was continued for four weeks in some cases but for as long as eight or nine weeks in many. Subsequent to this treatment certain patients were given an oral supplement of vitamin B-complex. Treatment with riboflavin was unsuccessful. It is of interest to note that one patient who had cheilosis showed no improvement of the rosacea even though the cheilosis cleared. Another patient who suffered an exacerbation of the rosacea during riboflavin therapy later developed cheilosis. This responded to the ingestion of riboflavin but during this time the rosacea once more flared up. Only one patient showed a reduction of symptoms and a decrease of signs of rosacea in association with riboflavin therapy. Even in this case the condition did not completely disappear.

These observations are contradictory to those of Johnson and Eckardt (*Arch. Ophth.* **23,** *899 (1940)*), who reported benefit following treatment with riboflavin. It is of interest that in contrast to the cases studied by Wise only 9 of Johnson and Eckardt's 36 patients had facial rosacea as well as rosacea keratitis. Furthermore, unlike Wise, Johnson and Eckardt frequently discovered this condition in Negroes. They observed that the condition which Wise calls marginal catarrhal ulcer responds quickly to riboflavin therapy, and it therefore seems probable that these investigators observed similar although actually different disorders.

In a later study, Conners, Eckardt, and Johnson (*Arch. Ophth.* **29,** *956 (1943)*) express the opinion that the association of facial lesions with ocular lesions in rosacea is purely coincidental. It is their experience that patients showing marginal corneal ulcers and catarrhal corneal infiltrates uniformly have a poor dietary intake of riboflavin, and by the microbiologic method of Snell and Strong the mean average daily excretion of riboflavin in the urine of these patients is about 60 per cent that found in the urine of normal controls. However, there is a large variation in these figures. These workers have found that the intravenous administration of 1 mg. sodium riboflavin per day for three to four days plus large amounts of vitamin B-complex by mouth produced prompt healing of the ocular lesions. They state that in their total experience they have encountered only one patient with typical superficial vascularization and superficial marginal infiltrates in the arcus senilis area who has not responded satisfactorily to this therapy.

The problems raised by the discrepancy between these two papers is well illustrated in a short paper by Fish (*Brit. J. Ophth.* **27,** *107 (1943)*) who subjected 45 patients with acne rosacea to biomicroscopic examination; the object was to determine whether these cases showed, in addition to the lesions usually described as rosacea keratitis, which are often unilateral, the bilateral superficial vascular proliferation described by Sydenstricker, Sebrell, Cleckley, and Kruse (*J. Am. Med. Assn.* **114,** *2437 (1940)*). Of these 45 patients all had skin lesions of rosacea, 43 had keratitis, and 40 had unilateral keratitis, thus offering the unulcerated eye as a control for comparison. The lesions characteristic of ariboflavinosis were not found in either eye of 40 subjects. Dr. Sydenstricker personally confirmed these observations. When riboflavin therapy was administered to 10 patients in the doses recommended by Eckardt and Johnson, no effect was noted. When atropine, dark glasses, and oral hydrochloric acid were added to the riboflavin in these 10 patients and 23 others, healing occurred within a short time. Ten patients receiving only the atropine, dark glasses, and hydrochloric acid showed equally satisfactory healing. The subject who showed the bilateral lesions of ariboflavinosis in addition to the keratitis of rosacea healed satisfactorily on riboflavin therapy alone. Accordingly, Fish believes that the keratitis associated with acne

rosacea is not coincidental, and that it is probably unrelated to riboflavin deficiency unless the bilateral corneal vascularization associated with that deficiency can be demonstrated. It is clear from Wise's careful study that the condition which he calls rosacea keratitis is unrelated to riboflavin deficiency. It is interesting in passing to note that Wise believes that rosacea is due to some factor, at present unknown, that produces prolonged vasodilatation of the facial vessels in the so-called blush area and that the other manifestations of this condition are the consequence of congestion, prolonged vascular dilatation, and secondary infection.

Thus the exact etiology of rosacea keratitis remains uncertain, and will remain so until there is more agreement on the criteria necessary for the diagnosis.

PLACENTAL TRANSFER OF VITAMIN C

Placental transfer of nutrients has been extensively studied during recent years and much interesting information has been accumulated to show how maternal nutrition affects that of the offspring. Practical use, for instance, has been made of the demonstration that vitamin K but not prothrombin can pass the placental barrier (*Nutrition Reviews* **1,** *104* (*1943*)). As another example, Gellhorn, Flexner, and Hellman (*Am. J. Obst. Gynec.* **46,** *668* (*1943*)), using radioactive sodium, obtained data which indicate that the rate of transfer of sodium across the human placenta increases markedly per unit of placental weight as gestation progresses. This increase can be correlated with the changing morphology of the placenta which consists of an increase in number and a decrease in cross sectional area of the villi, together with thinning of their walls and an increase in the number of fetal capillaries within the stroma of the villus. Review has recently been made of the investigations which show that probably carotene but not vitamin A can be passed through the placenta from mother to fetus (*Nutrition Reviews* **2,** *26* (*1944*)). It has also been established that the plasma vitamin C of cord blood or of the newborn infant is higher than that of the mother (Mindlin, *J. Pediatrics* **16,** *275* (*1940*); Javert and Stander, *Surg. Gynec. Obst.* **76,** *115* (*1943*)). Why this is so, if the process of transfer is one of simple diffusion, is difficult to understand.

Giroud *et al.* (*Compt. rend. Soc. de biol.* **121,** *1062* (*1936*)) suggested that the fetal values are higher because the fetus possesses the ability to synthesize vitamin C. An alternative explanation was made by Manahan and Eastman (*Bull. Johns Hopkins Hosp.* **62,** *478* (*1938*)) to the effect that selective filtration of ascorbic acid occurs. McDevitt, Dove, Dove, and Wright (*Proc. Soc. Exp. Biol. Med.* **51,** *289* (*1942*)) confirmed the observations of Manahan and Eastman and agreed that the placenta acts as a selective filter of vitamin C. An entirely new concept, supported in part by data, has been published by Lund and Kimble (*Am. J. Obst. Gynec.* **46,** *635* (*1943*)). This last study is given more detailed consideration.

Lund and Kimble also found that the plasma vitamin C value of cord blood was regularly higher than in maternal blood. When maternal demands for ascorbic acid were not met during pregnancy, the mothers became depleted at the expense of the fetus. For instance, only 7 of 94 babies had plasma values below 0.60 mg. per cent while nearly half of the mothers had values below that level. In only one instance did the maternal level exceed 1.40 mg. per cent but approximately 50 per cent of the fetal values were between 1.50 and 2.00 mg. per cent. However, the intravenous injection of ascorbic acid in

doses of 100, 250, and 500 mg. raised the plasma vitamin C of both maternal and cord plasma to almost identical levels when injections were made approximately two hours before delivery. Fetal plasma increased more slowly and did not reach an equilibrium with maternal values until approximately one to one and one-half hours after injection. When the time interval between the injection and delivery was greater than two hours, the maternal values had begun to decline but the cord level remained high for at least twelve hours. The fact that levels were approximately equal two hours after injection, however, suggested that simple diffusion and not selective filtration had occurred. The authors explain the more rapid fall in maternal levels by postulating that the placenta acts as a barrier to the reentrance of the ascorbic acid from the fetal to the maternal circulation. Maternal values fall because the vitamin C is utilized, excreted, or stored. The fetus utilizes ascorbic acid more slowly and has no method of excretion except through the placenta, and the placenta, it is suggested, selectively retains it. Presumably, transference of the vitamin from the mother is accelerated whenever the maternal level is raised, as after a meal. According to this concept, then, the idea of selective filtration is turned about, and to the placenta is ascribed the property of selective retention.

It must be pointed out that while this suggestion is extremely interesting, no direct evidence to prove its operation has been offered. Plasma ascorbic acid levels are notoriously labile and dependent on recent dietary intake. Determination of tissue vitamin C, or of the values in the white blood cell-platelet fraction of blood, would provide more convincing evidence of true maternal-fetal relationships. If these levels showed the same consistent difference found in plasma, it would be easier to accept the idea that the fetus takes its supply of the vitamin even if it must deplete the maternal organism.

BIOLOGIC VALUE OF MIXED PROTEIN IN FOOD

Our knowledge of the biologic value of protein has been obtained for the most part from experiments in which a single purified protein or the mixed proteins of an individual foodstuff were present in the diet. Little is known regarding the biologic value of the proteins making up a mixed diet in which the protein sources vary considerably. It is well recognized that the low biologic value of certain proteins, such as those from certain vegetable sources, can be considerably enhanced by the inclusion of proteins of animal origin in relatively small amounts. Thus in studies of the biologic value of certain grain proteins, Swaminathan (*Indian J. Med. Res.* **26,** *113 (1938)*) was able to raise the biologic value of rice proteins to as high as 90 per cent by the addition of relatively small amounts of skim milk. It is therefore becoming apparent that in appraising the actual biologic protein value of a mixed diet, it is often desirable to test the whole diet rather than to attempt to evaluate the contribution of the individual protein components.

Macrae, Henry, and Kon (*Biochem. J.* **37,** *225 (1943)*) have reported a study of the biologic value of mixed proteins in food served in Royal Air Force Stations in England. The total food available to 5 representative airmen at each of four stations was collected for one week, stored at −20°C. during the collection period, and then ground and dried. The protein consumed per man daily varied from 83 to 101 g., and the proportion of the protein which was animal in origin ranged from 42 to 45 per cent.

The dried material was used to determine the biologic values and true digestibilities of the diets according to the method of

Mitchell and Carman (*J. Biol. Chem.* **68,** *183 (1926)*). The diets were mixed with starch to give an 8 per cent level of protein (N x 6.25) intake, margarine to bring the total fat content to 10 per cent, and calcium acid phosphate to compensate for a calcium lack. The completed diet was mixed with one and a half times its weight of water and fed to three groups of 4 litter mate rats.

The results of the study reveal that the diets had a high biologic value and a high degree of digestibility. The biologic value, that is, the percentage of absorbed nitrogen which is not excreted in the urine, varied from 77 to 81. The true digestibility, based on the difference between the fecal nitrogen on a low protein diet (metabolic nitrogen) and the fecal nitrogen on the experimental diet, varied from 85 to 89 per cent. The "protein value" of the diets, calculated as the product of the biologic value and the true digestibilities, varied from 67 to 72.

Since none of the vegetable proteins attains the protein values of these mixed diets (a value of 77 for rice has been reported by Li [*Chinese J. Physiol.* **4,** *49 (1930)*] but most cereal proteins range from 50 to 65), it is reasonably evident that the admixture of 42 to 45 per cent animal proteins resulted in considerable supplementation of the poorer proteins by the better. The biologic value of these R.A.F. diets is surpassed only by the value of eggs and milk and is approximately equal to that of liver and kidney.

In the United States Army, the daily protein intake is estimated at 131 g. (Howe, *J. Am. Med. Assn.* **120,** *93 (1942)*), of which 62 per cent is of animal origin. It is a tribute to the personnel engaged in planning and providing the diet of the American soldier to realize that diets with protein values exceeding those recorded for the R.A.F. are provided for our men.

VITAMINS AND PHYSICAL FITNESS

There is great current interest in the relation of vitamin intake to fatigue and physical fitness, and it is unfortunate that a precise relation is most difficult to demonstrate. Measurements of physical fitness and ability to do work tend to be subjective; even attempts toward objective measurements have met considerable criticism. Experiments of this type require the most careful kinds of controls, and demand the nutritionist's most thoughtful criticism, since unjustified claims for the benefit of vitamins in these functions will receive all too quick acceptance by overenthusiastic vitamin therapists. A previous review concluded with the comment that hard physical work necessitates an adequate amount of the vitamin B-complex, but that excess vitamin intake over the generally accepted level does not produce any greater degree of physical ability.

In Great Britain, Harper, MacKay, Raper, and Camm (*Brit. Med. J.* **1,** *243 (1943)*) have also carried out experiments on the effect of vitamin supplements on physical fitness. The subjects were 69 cadets, 18 to 19 years of age. In addition to a physical examination and history of minor complaints, the resting heart rate, resting vital capacity, and R. A. F. breath-holding and endurance tests were performed. Following this the cadets were divided into two groups of 35 and 34 each. One group received a daily vitamin supplement of 6000 I.U. of vitamin A, 1000 I.U. of vitamin D, and 50 mg. of ascorbic acid. The other group received placebos. A history of minor complaints was taken every two weeks. After ten weeks the cadets were all reexamined in the same way as initially performed, and the vitamin supplements given to the other group, the first group now receiving placebos. This was continued for eleven weeks and the examination repeated.

Neither the cadets nor the examiners knew who was receiving vitamins. The results of the various measurements and tests were examined statistically. These authors report that the group receiving vitamins had an increased vital capacity, breath-holding time, endurance, and surprisingly, resting pulse rate. In addition, the control group had an incidence of minor gastrointestinal and respiratory complaints 50 per cent greater than that of the vitamin supplemented group. The authors conclude that these improvements with vitamin supplements are reflections of an inadequate dietary intake of vitamins A, D, and C, and that the physiologic tests employed are of value as a means of assessing an optimal vitamin intake.

Aside from the obvious criticism of the small number of subjects involved and the small differences noted, the question might well be raised as to the validity of the assumption that these cadets all had deficient diets to begin with, and further, as to whether the tests employed were really measures of physical fitness. Of the tests employed, the slow resting pulse rate is accepted by many as a measure of physical fitness and yet this was found to be higher in the vitamin fed group. The other tests have suffered some adverse criticism in their use as a measure of physical fitness (Schneider, "Physiology of Muscular Activity," *2nd ed., W. B. Saunders Co., Philadelphia (1939)*). Regarding vital capacity, it is accepted that this is increased with training, but "there is danger of overemphasizing vital capacity as a measure of fitness" (Steinhaus, *Physiol. Rev.* **13,** *103 (1933)*).

Jenkins and Yudkin (*Brit. Med. J.* **2,** *265 (1943)*) have reported a similar study on 178 children, 80 of whom were 12 year old girls, 76 were 12 year old boys, and 22 were 11 year old boys. About half in each group had received daily supplements of 5000 I.U. of vitamin A, 1 mg. of thiamin, 25 mg. of vitamin C, and 500 I.U. of vitamin D over a period of a year. The remainder received placebos. No statistically significant differences were noted between the groups when these were subjected to the same tests as used in the above study. The authors point out that this study does not necessarily contradict the findings of Harper *et al.*, since different age groups were involved and since there was no definite evidence of a deficient diet before the study was started. However, in the group studied by Harper and associates there was likewise no evidence of a deficient diet, but it was merely assumed that this was so. It was further brought out by the study of Jenkins and Yudkin that the vitamin supplemented group showed no improvement over the controls in gain in weight and height, strength of grip, hemoglobin, intelligence, educational attainment, or dark adaptation.

The subject of physical performance in relation to diet is critically reviewed by Keys (*Federation Proceedings* **2,** *164 (1943)*). While there is anything but agreement among the different reports as to the effect of the different vitamins on physical performance, the more careful studies fail to reveal any significant influence of vitamin supplements except in cases of definite deficiencies. According to Keys, the claims made by the Harvard workers (Johnson *et al., J. Nutrition* **24,** *585 (1942)*) that a diet deficient in vitamin B-complex results in physical deterioration following bouts of hard work, "must be rejected in toto." This conclusion is based on Keys' evaluation of the experimental setup employed by the Harvard group, in addition to his own experiments designed to evaluate this relationship. In regard to vitamin A, there appears to be no reliable evidence that normal muscular function is in any way related to vitamin A intake. Keys reviews a number of papers which tend to support the generalization that ascorbic acid is without benefit on the work capacity of individuals free from clinical scurvy.

Some of the discrepancies between the results of Keys' laboratory and the Fatigue Laboratory at Harvard might be explained on the basis of different experimental conditions. The level of intake of B-complex vitamins was lower in the Harvard experiments than in those of Keys, as judged by a more rapid decline of urinary excretion of thiamin to lower final levels. The Harvard group worked hard at a variety of outdoor tasks in a New England January; Keys' subjects worked on a treadmill indoors at 75°F. It will be recalled that Ivy (*Nutrition Reviews* **1,** *407 (1943)*) reported conclusions similar to those of the Harvard group.

It would appear that the evidence at hand indicates that supplementation of a "normal" diet is without benefit in improving physical efficiency, assuming of course that the normal diet is adequate. No one denies that deficiency of any nutrient results sooner or later in physical deterioration, but how low the dietary intake of specific nutrients can be without evidence of physical deterioration, and what the clinical syndromes of very early deficiency may be are still unsettled.

THE RICE PROBLEM

One of the most important public health problems in India, Malay, East Indies, and the Philippines, where rice is a staple food, is that of finding practical methods for prevention of beriberi and the concomitant dietary deficiencies prevalent among the vast poor sections of the population. Even though the causes of this condition (rice eaters' disease) have been known for years, it continues to be responsible for invalidism, disability, and enormous loss of life.

A completely satisfactory solution to the rice problem is dependent upon the adjustment of many difficult social, economic, and agricultural conditions which may require years or even generations to accomplish. In the meantime, the development and application of sound nutritional knowledge in the processing and preparation of the available food supply promises at least an improvement over conditions as they now exist.

A number of papers have appeared from the Nutrition Research Laboratories at Coonoor, South India, dealing with the problems of how to obtain the maximum nutritive value from rice. The rice problem in India has been reviewed by Aykroyd, Krishnan, Passmore, and Sundararajan (*Indian J. Med. Res., Memoir no.* **32** *(1940)*). Since then papers by Swaminathan (*Indian J. Med. Res.* **29,** *83 (1941)*; **30,** *409 (1942)*) have contributed additional interesting data.

The wide use of polished (white) rice in the Orient developed for many of the same reasons that have made white flour popular in the Western countries. The keeping qualities of this highly refined cereal were found superior to those of a less well refined product. Subsequently, a popular demand arose for the bland white form.

The cortical (external) portion of rice, wheat, and other grains is rich in minerals and a number of the B-vitamins, while the endosperm (central part) contains very small amounts of these substances and is composed mostly of carbohydrate (starch). Therefore, when highly polished rice constitutes a major part of the diet, the vitamins and minerals of the whole grain are lost. Usually insufficient quantities of other foods are consumed to compensate for this loss, and widespread malnourishment results.

Aykroyd and Swaminathan (*Indian J. Med. Res.* **27,** *667 (1940)*) have shown that parboiling of rice (steaming in the husk) greatly reduces the losses of thiamin and nicotinic acid from the grain on subsequent milling. Parboiled, milled rice contains three to four times as much of these factors as raw rice of the same botanic variety

milled and processed to the same degree. This is interesting in relation to the epidemiology of beriberi, for it has been long known that this deficiency is almost nonexistent in those localities where it has been the custom to parboil the rice before milling. It appears that steaming leaches the vitamins from the hull which are then absorbed into the endosperm and, therefore, not lost upon milling. Further studies have shown that while raw milled white rice loses 60 per cent of its thiamin and nicotinic acid during the washing process preparatory to cooking, only 8 to 12 per cent of these substances are lost when parboiled rice is washed in a like manner. Approximately 25 per cent of the vitamins in either raw or parboiled rice is dissolved out with the cooking water. These investigations show that the combined procedures of processing, washing, and cooking of polished rice lead to a loss of nearly all of the thiamin and nicotinic acid, while cooked parboiled rice, even when the cooking water is discarded, contains enough thiamin to prevent the occurrence of beriberi. The following table summarizes these observations:

	RAW MILLED	PARBOILED MILLED
	micrograms per gram	*micrograms per gram*
Thiamin content.....	1.0	2.1
After washing.....	0.4	1.9
After cooking.....	0.25	1.4
Nicotinic acid content..	1.6	3.2
After washing.....	0.65	2.83
After cooking.....	0.39	1.56

It can readily be seen that if parboiling of rice before milling can be made a practical, acceptable procedure, great benefits would result to a large portion of mankind. Steaming before milling introduces an extra step and increases the cost in processing. However, during the milling of ordinary white rice a significant loss due to fracturing of the grain occurs, and these broken grains are diverted to animal feeds. It is claimed that the loss from this cause is much less with parboiled rice, and the resulting saving may be sufficient to offset the cost of steaming.

One objection to parboiled rice in the past has been the musty flavor often present. This is due to lack of the proper technical control necessary for the production of a dry product. It has now been shown that this musty flavor can be prevented. Parboiled rice (called converted rice) is being produced on a limited commercial scale in this country and in England. But since most of it is used by the armed forces, it has not reached the retail markets in quantity.

Attempts to apply the steaming method to wheat as a means of producing an enriched flour have failed. The heating process appears to change the wheat protein so that it is no longer suitable for bread making.

The potential benefits to be derived from the wide use of parboiled rice can be realized when it is considered that nearly half of mankind depends upon this cereal for from 70 to 80 per cent of its food. The application of this technical improvement in the processing of rice could easily become one of the most practical contributions of the science of nutrition to human welfare.

THE MEDICAL APPRAISAL OF CALCIUM NUTRITION

Every study of dietary habits and food intake in this country furnishes evidence that a large proportion of the population ingests less calcium than it needs according to recommended standards. Sherman and Lanford ("Essentials of Nutrition," *The Macmillan Co., New York (1940)*) have collected impressive evidence from chemical balance studies on human beings and from animal experiments to indicate that one of

the directions for improvement of nutritional well-being and resultant better health is an increase in calcium intake. In contrast with these findings is the rare occurrence of calcium deficiency which can be detected by clinical methods. The classic diseases which are due to lack of this mineral are the tetanic complications of rickets in the child and osteomalacia in adults, but in both conditions the adequacy of the supply of vitamin D often overshadows the role of calcium deficiency.

Attempts to develop methods to detect early and mild degrees of calcium deficiency have met with little success. Youmans and Patton (*Clinics* **1,** *303 (1942)*) state that the use of the x-ray to detect early or slight demineralization of the skeleton is as yet unsatisfactory and unreliable. These authors have found that the use of a photoelectric cell device with a reading galvanometer to measure the density of bone on roentgen films, in comparison with a standard density on each film, does not solve the problem. They also emphasize that the x-ray cannot distinguish the osteoporosis due to calcium or vitamin D deficiency from that resulting from other causes.

Beebe (*Milbank Memorial Fund Quart.* **21,** *311 (1943)*) has studied the possibility of using the neuromuscular response to galvanic current as a guide to the adequacy of calcium nutrition, following up the well known alterations in this response which constitute Erb's test for latent tetany. The degree of stimulation of the ulnar nerves of both arms required to produce the least visible twitch, a visible finger twitch, and entire hand twitch, was determined by means of a standardized procedure in 1800 adolescent New York schoolchildren. The results obtained on each arm were interchangeable, and the data on the right arm as milliamperes of current required to produce a least twitch as cathodal opening stimulus and cathodal closing stimulus, and a visible finger twitch as anodal closing stimulus and cathodal closing stimulus, were used for statistical analysis.

The results show some variation with age and a consistent sex difference with definitely lower values for girls. A seasonal variation was observed, with a lower threshold of neuromuscular stimulation apparent in the late spring than in early fall or winter. No attempt was made to determine if this temporal variation was due to physiologic variability or to technical errors beyond control, but it was concluded that error is so large a part of any observed variability that no useful clinical purpose can be served by a single galvanometric determination on a subject.

In exploring the correlation between the threshold of neuromuscular stimulation and the dietary calcium intake, attempts were made to control variations such as serum calcium levels and the degree of skeletal maturation by dividing the subjects into groups. Dietary calcium was estimated from two-day consumption records which indicated that 72 per cent of the subjects were ingesting less than the recommended daily allowance for their age and sex, while 28 per cent received even less than two thirds of the recommended amount.

The measurement of neuromuscular irritability used was found to be statistically independent of the dietary calcium intake. Correlation of the level of total serum calcium with the threshold of galvanic stimulation yielded values at the level of borderline statistical significance in only four of the fifty-six groups into which the data were divided. The level of ionized calcium in the serum was unfortunately not estimated. Of interest is the finding that the calcium intake and the level of serum calcium also appeared entirely unrelated. The skeletal age was estimated from roentgenograms of the wrist and hand, elbow, and hip, from the Western Reserve University standards of Todd, and compared with chronologic age. Three degrees of skeletal maturity were established for each age, and

correlation of these with the level of neuromuscular irritability showed only chance association.

Beebe suggests that the metabolic situation in simple calcium deficiency may differ qualitatively rather than quantitatively from that in tetany, and cites animal experimentation in which a lowered neuromuscular response to galvanic stimulation was not produced until the dietary calcium to phosphorus ratio was lowered to 1:5.6. Such a ratio is not likely to occur in human dietaries because of the association of calcium and phosphorus in the same foods. There is adequate reason for regarding tetany, manifest or latent, as due to a breakdown in the body's homeostatic mechanism for maintaining a uniform level of ionized calcium in the blood. Such a breakdown, in the mildest degree, caused by inadequate dietary intake, would be expected to occur only after physiologic compensatory mechanisms had been exhausted, and so would be a late, not an early, evidence of calcium deficiency.

Tedious chemical balance studies therefore remain as the only means of definitely demonstrating early degrees of calcium deficiency, and even these are affected by endocrine and dietary factors.

CORNEAL VASCULARIZATION IN VITAMIN DEFICIENCIES

The observation by Bessey and Wolbach (*J. Exp. Med.* **69,** *1 (1939)*) that vascularization of the cornea occurs early and constantly in albino rats that are riboflavin deficient and the subsequent report by Sydenstricker, Sebrell, Cleckley, and Kruse (*J. Am. Med. Assn.* **114,** *2437 (1940)*), that similar changes were to be found in malnourished patients with cheilosis have aroused much interest in this ocular finding. Many investigators have assumed that corneal vascularization is a specific sign of riboflavin deficiency. Mainly on this ground, for example, it was reported that riboflavin deficiency existed in as many as 75.8 per cent of the pupils in a school in the east side of New York City (Wiehl and Kruse, *Milbank Memorial Fund Quart.* **19,** *241 (1941)*).

A number of physicians have expressed doubt as to the specificity of corneal vascularization as a sign of riboflavin deficiency and have waited for a critical evaluation of the question by an ophthalmologist. Such a special study has been reported by Vail and Ascher (*Am. J. Ophth.* **26,** *1025 (1943)*).

Working in the Department of Ophthalmology of the University of Cincinnati, as well as in the Nutrition Clinic in Birmingham, Alabama, over a period of three years, Vail and Ascher base their report on an extensive study of the corneas of 711 patients. The report begins with a detailed account of the normal vascular arrangements in the cornea, conjunctiva, and sclera. These workers point out that there is a normal pericorneal plexus of vascular arcades which are so located that their final loops seem to be in the corneal tissue when they are really only in the transparent tissue that is the conjunctivoscleral wedge. These loops frequently are entirely free of blood corpuscles although most likely they are patent at all times and perhaps may contain blood plasma or plasma diluted with aqueous humor. Hyperemia from any cause results in the filling of some or all of these capillaries with red cells so that they become apparent when previously they were practically invisible. Vail and Ascher insist that actual vascularization of the cornea occurs only where pathologic changes have taken place in the cornea. They would reserve the term vascularization for actual proliferation of blood vessels. They state that Sydenstricker's conclusion that a "normal avascular zone between the limbic plexus and the sclerocorneal junction is

overrun by minute vessels from which spring capillary loops" is a misconception and that what he described was produced by a filling of capillaries normally present in the limbic plexus. They propose the term "concentric collaterals" for the vascular changes observed since the condition described as corneal vascularization is only an engorgement of the preexisting limbic plexus.

During the summers of 1940 and 1941 Vail and Ascher studied 642 patients in the Birmingham Nutrition Clinic who had deficiency disease of various degrees of severity. Of these patients 85 were observed to have concentric collaterals but in not a single case was true corneal vascularization found except where previous corneal disease, as evidenced by infiltrations, ulcers, or scars could be seen. Thus concentric collaterals were observed in 13.3 per cent of the Birmingham group of patients.

More exact studies were made of 44 patients who were regarded, by the physicians of the Nutrition Clinic, as having vitamin deficiency. Of these, 12 were regarded as having riboflavin deficiency, 13 niacin deficiency, 2 thiamin deficiency, 8 "subclinical" deficiency, and 13 "questionable deficiency." Of the total number 32 patients were aware of signs and symptoms of conjunctivitis. Photophobia was present in more than one half of the patients and hemeralopia in about one fifth of them. Objectively none was free of signs of conjunctivitis, follicular hypertrophy, corneal scars, pterygium, or pingueculae.

For comparison with patients seen at the Nutrition Clinic, 69 patients were examined in the Outpatient Eye Clinic of the University of Cincinnati. These patients were taken at random from the patients coming for eye refraction except that those with acutely inflamed eyes and those with corneal disease were excluded. Thirty-one of the 69 patients showed typical, complete, concentric collaterals in one or both eyes and 6 others showed a larger amount of incomplete concentric collaterals. Thirty-two patients showed only a few or no limbic loops filled with blood.

The dietary histories of these patients were studied carefully and the patients were divided into three groups on this basis. Those classified as having a good diet drank at least 2 pints of milk daily, had at least 2 helpings of vegetables daily, at least 1 egg and 1 square of butter daily, carrots at least once a week, fruits at least once a day, and meat daily. Those classified as having a poor diet took less than half a pint of milk daily, less than 1 egg daily, less than 1 square of butter daily, less than 1 vegetable daily, and meat less than three times a week. Twenty-four patients were classified as receiving a good diet and 28 as taking a poor diet. There were 17 whose diets took an intermediate position between those classified above as good and poor.

The study showed that a larger proportion of the patients taking a good diet were found to have concentric collateral vessels than those taking a poor diet. Of those taking a good diet 14 showed collateral vessels and 10 showed none. Of those taking a poor diet 11 showed collateral vessels and 17 showed none. Of the intermediate group of 17 cases, 12 were found to have concentric collaterals and 5 showed none. It is also to be noted that the percentage of eyes with concentric collaterals was higher in the Cincinnati Eye Clinic group (45 per cent) than in the Birmingham subjects (13 per cent). This is just the reverse of what one might expect were the presence of these vessels indicative of vitamin deficiency.

Vail and Ascher conclude that what Sydenstricker termed corneal vascularization "is nothing more than extensive engorgement of the preexisting limbal meshwork, except in cases of obvious corneal disease." They deny that there is a "normal avascular zone" between the limbic plexus and the sclerocorneal junction. They point out that it is only the anatomic arrangement in the conjunctivoscleral wedge which makes this

region particularly easy to illuminate from behind, as is done in biomicroscopy. Observed by this means, they say, concentric collaterals contained in the tissue overlapping the corneal rim appear to be situated in the cornea proper and may be mistaken for real corneal vascularization when they represent merely a special kind of engorgement in the marginal meshwork. They conclude that concentric collaterals are the sequela of an extensive conjunctival congestion of long duration or of repeated occurrence. Any form of conjunctivitis may lead to congestion in the limbic meshwork. To regard this reaction as a specific sign of riboflavin deficiency does not seem to be justified although it is not necessarily denied that such deficiency may sometimes be the primary or secondary cause.

It has been stated that riboflavin deficiency is the most common type of vitamin deficiency to be found in this country. The chief basis for this statement is the finding of corneal vascularization such as Sydenstricker and co-workers described. If the work of Vail and Ascher is confirmed, the criteria for biomicroscopic estimation of the prevalence of riboflavin deficiency, which some workers hold, will have to be greatly revised.

THIAMIN AND RIBOFLAVIN ENRICHMENT

That the present day human dietary is frequently deficient or dangerously low in various important members of the B-group of vitamins is generally recognized. Indeed, it was this recognition that led to the initiation of the food enrichment program now becoming firmly established in this country. Because of the significance of the results in relation to the enrichment of white flour and bread, clinicians and nutritionists will find interest in some experiments from the Mayo Foundation laboratories.

Early in 1943, Higgins, Williams, and Mason (*J. Nutrition* **25,** *229 (1943)*) discussed some observations on rats fed a human diet which was low in thiamin. The basal diet contained food items common in many human diets, and included beef roast, gelatin, string beans, potatoes, cheese, dry skim milk, carrots, rice, corn flakes, sugar, candy, apple jelly, butter, cream, peas, peaches, cake, iodized salt, and mineral and vitamin supplements. Bread was also furnished, the four diets differing in the flour used to make the bread. The thiamin-low diet contained bread made from white patent flour; other diets contained bread in which the thiamin or both thiamin and riboflavin were restored to whole wheat level, and bread in which whole wheat flour itself was used.

In a later paper, Higgins, Williams, Mason, and Gatz (*J. Nutrition* **26,** *347 (1943)*) report an extension of these experiments, in which the diets were low in both thiamin and riboflavin. In these studies, it was necessary to omit skim milk powder and cheese from the diet, larger proportions of some of the food items were allowed, and certain fruit and cereal items were substituted. In general, however, the basal diet resembled that used in the previous study. Diets were fed in which the flour making up the bread consisted of (1) patent white flour, (2) white flour containing thiamin at the level in whole wheat, (3) a similar flour in which both thiamin and riboflavin were restored to whole wheat levels, (4) a restored flour to which 6 mg. of niacin per pound of flour was added, and (5) whole wheat flour. The most deficient diet contained 0.96 microgram of thiamin and 2.0 micrograms of riboflavin per gram of dry matter; the diet containing whole wheat bread furnished 1.94 and 2.60 micrograms of thiamin and riboflavin, respectively. Groups of 8 rats were fed ad libitum for fourteen weeks on each of the above diets.

In general, the results of the two experiments cited are in agreement. Thiamin was found to have only a slight influence on the growth rate, whereas riboflavin very significantly increased the rate of growth of the animals. This rate was not appreciably altered by the further addition of niacin to the patent flour. Whole wheat as a component of the bread induced a much greater growth rate than was obtained from any of the other diets, despite the inclusion of three B-vitamins at whole wheat or enrichment levels in the flour, and despite a somewhat smaller food intake for rats receiving whole wheat as against those receiving the enriched patent flour. In the earlier studies reported by Higgins and co-workers the rats received half again as much riboflavin as in the later experiments, and growth was considerably better than the 1 g. per day rate observed in rats receiving whole wheat having a lower riboflavin content.

The test animals on the basal deficient diet developed a hypochromic anemia which was only slightly improved by restoring the thiamin to the patent flour. Significant increases in both the erythrocyte and hemoglobin levels in the blood were observed when both thiamin and riboflavin were added to the basal diet. The use of whole wheat rather than enriched white flour further improved the hemoglobin picture, but did not alter the production of erythrocytes.

Similarly, disturbances were noticed in the livers of the animals on the thiamin- and riboflavin-deficient ration. Extensive peripheral necrosis of the cells was observed, together with some fatty degeneration of the peripheral portions of the nodules. Cirrhosis did not develop, however. Although both thiamin and riboflavin improved the condition of the hepatic cells, only on the diet containing whole wheat was the appearance of the liver section nearly normal.

In all of the experimental animals and without regard to the test diet fed, the thyroid glands were hyperplastic. The authors cannot explain the hyperplasia on the basis of an iodine deficiency; at least it seems certain that this condition is not related to the experiments concerned, since even the rats receiving whole wheat bread showed extensive cellular hypertrophy.

Thiamin and riboflavin analyses on the tissues of rats on the control laboratory ration and on the diet deficient in thiamin and riboflavin demonstrated the effect of inadequate diet in depleting the tissues of these factors. The concentrations of thiamin in the liver, skeletal muscle, kidney, and testes were lowered in a striking manner when the diet contained unenriched flour. A similar picture was observed for riboflavin in the liver and kidney, but no lowering of the riboflavin concentration was evident in muscles or testes. Hepatic tissue changes and body growth were both significantly benefited by enrichment of the flour with the B-vitamins, although to a lesser extent than when whole wheat itself was used in the bread.

The authors conclude that "fortification of the flour used in the bread component of the low thiamin-low riboflavin human diets with thiamin, riboflavin, and niacin in the amounts indicated proved inadequate to promote satisfactory growth or to prevent pathologic changes in the livers, thyroids, and pituitary glands of white rats." In view of this observation, it will be of interest to observe the clinical results of a similar dietary study on man (*J. Nutrition (in press)*). Experiments along these lines should be of great value in guiding future trends in the enrichment program.

DESTRUCTION OF ASCORBIC ACID IN THE GASTROINTESTINAL TRACT

The possible destruction of vitamins in the intestinal tract has been overshadowed by the recent findings that several vitamins may be synthesized by bacteria of the intestine and utilized by the host. However, at least one of the vitamins, ascorbic acid, does not appear to be synthesized by intestinal bacteria but rather destroyed by a wide variety of organisms.

Cases of clinical scurvy have been reported which fail to respond normally to oral ascorbic acid therapy, but which are cured by intramuscular injections. Several years ago Einhauser (*Z. f. d. ges. exp. Med.* **98,** *461 (1936)*) reported that conditions of hypoacidity and gastroenteritis were accompanied in some instances by disturbed absorption and destruction of the vitamin. It was suggested that the destruction was due to the high pH of the intestinal contents as well as to the growth of intestinal bacteria in the stomach. In this connection it is of interest that Dyke *et al.* found that patients with pernicious anemia showed better therapeutic responses to liver therapy when high doses of vitamin C were given (*Nutrition Reviews* **1,** *108 (1943)*).

The destruction of ascorbic acid by Escherichia coli has been reported by Stepp and Schröder (*Klin. Wchnschr.* **14,** *147 (1935)*), and destruction by members of Mucosus capsulatus and Enterococcus groups has been shown by Kendall and Chinn (*J. Infect. Dis.* **62,** *330 (1938)*). Studies on the destruction of ascorbic acid by human fecal cultures have already been reviewed (*Nutrition Reviews* **1,** *86 (1943)*).

The problem of vitamin C destruction by bacteria has been further investigated by Young and Rettger (*J. Bact.* **46,** *351 (1943)*). These authors made a general survey of the ascorbic acid decomposing ability of members of various genera of Eubacteriales. In addition, studies were carried out on the influence of various carbohydrates on vitamin C destruction, the relation between bacterial growth and stability of the vitamin in different mediums, and the mode of decomposition of ascorbic acid by the bacteria. It was found that members of the following genera oxidize ascorbic acid: Escherichia, Aerobacter, Salmonella, Erbethella, Streptococcus (enterococci and hemolytic), Encapsulatus, and Vibrio. With two exceptions, it was observed that when one species in a genus was capable of destroying ascorbic acid, all the other members of the genus possessed this property. It was interesting to note that when an organism attacked the vitamin at all, the decomposition was complete. Two strains of Proteus morgani were active in destroying ascorbic acid, while three strains of Proteus vulgaris were inactive. Of eight species of Salmonella studied, Salmonella pullorum alone failed to destroy the acid.

Studies on the influence of different carbohydrates on vitamin C decomposition revealed that in the presence of a rapidly fermentable carbohydrate, no destruction occurred. When the carbohydrate was not fermented at all, or only slowly, rapid decomposition of the ascorbic acid resulted. The carbohydrates studied included glucose, lactose, xylose, sucrose, dextrin, and mannitol.

Ascorbic acid was found to serve as a ready source of carbon for the attacking bacteria when the medium contained organic nitrogen, such as peptone. This was evidenced by the increased bacterial growth and the simultaneous loss of the vitamin in the medium.

A study of the mode of decomposition of ascorbic acid revealed that in every instance the oxidation was carried beyond the first stage and thus was irreversible. The oxidation was carried out only by actively metabolizing cells, filtrates being completely inactive. From studies using various

enzyme inhibitors, and dye-carrier systems with resting cells, the authors suggest that a specific ascorbic acid dehydrogenase system is present in those organisms found to destroy vitamin C.

The results of the preceding studies indicate that under certain gastrointestinal conditions, ascorbic acid deficiency may result due to bacterial destruction. In cases of achlorhydria, the additional mechanism of ready aerobic oxidation at high pH may operate. It is conceivable that in the absence of large intakes of ascorbic acid in the diet factors such as alkalinity of the gastrointestinal tract (due either to achlorhydria, or to the popular practice of alkalinization) and changes of intestinal flora to one where ascorbic acid destroying organisms predominate, might lead to deficiency symptoms. While the latter may not represent those seen in frank scurvy, they may be responsible for delayed wound healing.

PANTOTHENIC ACID DEFICIENCY IN SWINE

Pantothenic acid deficiency has been produced in the chick, rat, mouse, dog, and pig, but the manifestations of the deficiency have been somewhat different in the various species. A rather complete account of the manifestations of pantothenic acid deficiency in swine has been reported by Wintrobe *et al.* (*Bull. Johns Hopkins Hosp.* **73**, *313 (1943)*). As in other experiments reported by these workers, young pigs 3 weeks of age were fed a basal diet of casein, sugar, and lard supplemented with a complete salt mixture, vitamins A and D, and the crystalline B-vitamins. The animals in this series received adequate thiamin, riboflavin, nicotinic acid, pyridoxine, choline, p-aminobenzoic acid, and inositol. Pantothenic acid was either completely omitted or given in inadequate amounts.

The effects of deficiency became evident as early as eleven days following commencement of the experiment. Diarrhea appeared, the pigs lost their appetite, their coats became rough and their hair unkempt. So rapidly did the deficiency develop and progress that the appearance of the pantothenic acid deficient pigs contrasted not only with the well-rounded lively pigs with smooth coats whose diets contained calcium pantothenate, but even with that of pigs whose diet was deficient in thiamin or in pyridoxine. In the latter two types of deficiency, more time was required for the effects of deficiency to become manifest and even then the visible evidences of deficiency were less pronounced than in pigs lacking pantothenic acid.

The impairment of growth in these animals was extraordinarily pronounced. The average daily weight gain was as low as zero to 92 g. per day in pigs given subminimal amounts of calcium pantothenate as compared with approximately 433 g. per day in control animals. In general the average daily weight gain was poorer in pigs given inadequate amounts of calcium pantothenate than in those given none at all. This apparent paradox is explained by the earlier death of animals given none of this vitamin.

In contrast to what has been seen in black rats, graying of the hair in black pigs did not occur. The hair became very thin, however, and fell out. A patchy alopecia was especially pronounced over the rump.

Rectosigmoidoscopic examinations were made from time to time in these animals since diarrhea was so prominent a symptom. The earliest change noted was the development of edema of the bowel. Soon this was followed by hyperemia and an exceptional tendency to bleed following the slightest trauma, as for example by gentle swabbing of the bowel. Eventually the rugae of the bowel disappeared. In the fully developed condition, the bowel appeared congested and edematous. The rectum showed the

most pronounced changes, but the bowel could be seen to be affected for the full 25 centimeters which could be observed through the rectosigmoidoscope.

Histologically, the essential change seemed indicative of injury to the cells lining the glands of the colon. The mucous vacuoles disappeared and the cells became atrophic. At first occasional glands throughout the bowel showed leucocytes in their lumina and in the periglandular interstitial tissue. In the more advanced stages of deficiency, however, more and more glands showed this change. The lumina of the glands finally became filled and overdistended with polymorphonuclear leucocytes while the lining epithelium became atrophic and flattened. Coincident with these changes, lesions were found in the lymphoid tissue of the submucosa. These were interpreted as the consequence of destruction of mucosal cells with the formation of abscesses in the prolongations of glandular elements which are normally found in the solitary lymphoid follicles.

Other changes included reddening and scaliness of the skin, patchy areas of red at the margins of the tongue, and the development of cough accompanied by a watery discharge from the snout. As previously reported by these workers, the gait became abnormal and histologic examination of the nervous system revealed degeneration in the sensory neuron.

A moderate normocytic anemia developed fairly consistently accompanied by a fall in the plasma chlorides. Less regularly the carbon dioxide combining capacity of the blood tended to rise. In extreme stages of deficiency, the nonprotein nitrogen of the blood was increased. The severe hypoglycemia observed in dogs (Schaefer, McKibbin, and Elvehjem, *J. Biol. Chem.* **143,** *321 (1942)* was not a feature in the pigs.

The condition described in these animals resembles in many ways the "pig pellagra" which has been reported as responding to treatment with nicotinic acid. The authors point out that in the experiments reported heretofore, the development of "pig pellagra" has occurred when other essential foodstuffs, such as proteins and other B-vitamins, were lacking as well as nicotinic acid. They state that in their own experience, when only nicotinic acid was lacking in the diet, diarrhea did not develop. They suggest that the exact pathogenesis of "pig pellagra" and of "necrotic enteritis" needs reevaluation.

The authors also suggest that the changes observed in the bowel in pantothenic acid deficiency may be due to the absence of mucous secretion or perhaps are caused by the lack of some enzyme or enzymes normally secreted by the colonic epithelium. The extremely pronounced impairment in growth rate is perhaps entirely due to the continuous diarrhea and loss of appetite, but a specific effect on growth is not excluded.

It has been suggested that there may be an alteration in secretion of adrenal cortical hormone in pantothenic acid deficiency (Supplee, Bender, and Kahlenberg, *Endocrinology* **30,** *355 (1942)*). Wintrobe and co-workers conclude that it is not necessary to make such an assumption in order to explain the chemical changes which are found in the blood of pigs deficient in pantothenic acid. They point out that when the serum chlorides fell in their animals, no sodium was lost in the urine. This is in contrast to the findings in Addison's disease. The drop in serum chlorides which accompanies pantothenic acid deficiency is more likely the result of inanition and diarrhea, while the rise in the carbon dioxide combining capacity of the blood is probably a compensating change for the drop in serum chlorides. The sharp rise in nonprotein nitrogen in the terminal stage of deficiency may be due to renal failure. Consistent with their view is the fact that they were unable to find any pathologic changes in the adrenal glands.

NOTES

LETTERS TO THE EDITOR

Dear Sir:

The person who wrote the review of my papers dealing with "Temperature Effects upon Vitamin Requirements" (*Nutrition Reviews* **1,** *426 (1943)*) not only failed to stress, but actually beclouded, the most important point of the work so far as it may relate to human nutrition. Vitamins are normally obtained in natural foodstuffs, and my studies clearly show that best growth response requires twice as much thiamin per kilo of food in tropical heat as in temperate coolness. It may be of interest to nutritional investigators that this actually calculates out to almost the same amount of thiamin per gram of weight gain in the heat and cold, due to the fact that food consumption in the heat is only 70 per cent of that in the cold. So far as practical nutrition is concerned, however, it means that an individual in tropical heat must eat food twice as rich in thiamin if he wishes to cover his optimal requirements. Cowgill's idea of a definite thiamin/calorie ratio is valid only for cool climate conditions.

With choline the reviewer also beclouded the importance of a greatly heightened need in tropical heat by his references to satisfactory rat growth with choline-free diets (work done in cool climates only).

My experiments were designed primarily toward clarifying the problems and handicaps of tropical nutrition. It was only after thorough consideration of the matter that I decided upon addition of the vitamins to the diets as most nearly approximating real-life problems of food intake. This approach evidently failed to satisfy a number of people, but it does have its good points for the purposes of this particular study.

Very truly yours,

Clarence A. Mills,
Professor of Experimental Medicine,
University of Cincinnati,
Cincinnati.

Dear Sir:

In your interesting article "Fetal-Maternal Relationship of Vitamin A and Carotene" (*Nutrition Reviews*) on pages 26–28 of the issue of January 1944, there appears the statement "Dann (*Biochem. J.* **26,** *1072 (1932)*) . . . believed the placenta impervious to carotene. . . ."

This is scarcely correct, and the only statement about such permeability which appears in the paper cited is as follows: ". . . whenever vitamin A was found in the fetal liver the assumption was made that this vitamin was obtained from the mother by transmission through the placental barrier. Two other possibilities demand attention. First, it is possible that carotene and not vitamin A passes from the mother to the fetus; a possibility which cannot at present be tested with the rat. . . ."

Very truly yours,

W. J. Dann,
Associate Professor of Physiology,
Duke University School of Medicine,
Durham.

Comparison of Thiamin Assay Methods for Flour and Bread

The vitamin B_1 subcommittee of the Accessory Food Factors Committee of the Medical Research Council and the Lister Institute have reported (*Biochem. J.* **37,** *433 (1943)*) the results of an investigation comparing different methods for determining thiamin in flour. Chemical, biologic, and microbiologic assays were carried out on specimens of national flour, as well as on a sample of bread made from one of the flours. The rat growth and bradycardia methods were compared with the yeast fermentation method of Schultz, Atkin, and Frey and these in turn with two chemical procedures. The latter methods were (1) the thiochrome procedure, in which it was concluded that

the photoelectric measurement was no more accurate than the visual, and (2) the p-amino acetophenone diazotization procedure of Platt and Glock (*Biochem. J.* **37,** *439 (1943)*), which was found reliable and well adapted to routine determination of thiamin in flour.

The samples were analyzed by fifteen independent investigators employing one or more of the assay methods, and their results evaluated statistically. There was general agreement of values obtained with the different methods. In general, the chemical procedures offer the simplest and most accurate determination of thiamin in flour and bread.

"Fish Disease" in Man

The syndrome Chastek paralysis in foxes is known to be associated with the consumption of raw fish. The similarity of the symptoms to those of thiamin deficiency aroused interest in nutritional circles and culminated in the finding that raw fish contains a heat labile factor which destroys thiamin. These developments have been previously reviewed in some detail (*Nutrition Reviews* **1,** *61, 403 (1942–43)*).

It is inevitable that the question be raised concerning the possibility of the occurrence of a similar syndrome in man from the same cause. Owen and Ferrebee (*New Engl. J. Med.* **229,** *435 (1943)*) have effectively dispelled this possibility by pointing out the special circumstances under which the fox disease is produced. They note that the thiamin destroying factor occurs mostly in the viscera of certain fish, less in the heads, skin, and scales, and least in the muscle. The portions edible for man therefore contain little of the factor. Furthermore, the agent is destroyed by cooking and by peptic digestion, and it has been shown that the consumption of raw fish as a separate meal is harmless. In feeding foxes it was common practice to mix the raw fish with other components of the diet prior to feeding, during which time the destruction of thiamin in the whole diet occurred.

Tomatoes as a Source of Ascorbic Acid

Tomatoes are secondary only to citrus fruits as a common source of dietary vitamin C. A field grown, fully vine ripened tomato may contain approximately 25 mg. of ascorbic acid, or nearly half the generally accepted daily requirement of vitamin C. This is the ideal tomato; those often found in markets, particularly those available in the early spring and late fall, are much poorer than the ideal.

Emphasis has been given to the inadequacy of late winter tomatoes by the report of Holmes, Jones, and Ritchie (*New Engl. J. Med.* **229,** *461 (1943)*). Thirteen lots of such tomatoes were carefully analyzed for ascorbic acid and found to range from 2.5 to 22.0 mg. per 100 mg., the average containing 8.8 mg. This average is much less than the 14 to 15 mg. per 100 mg. found in tomatoes which had been canned at the peak of the season (Daniel and Rutherford, *Food Research* **1,** *341 (1936)*). In terms of cost for the daily vitamin C requirement, late winter tomatoes represent a poor investment. The average cost of 75 mg. of acsorbic acid from this source was 72 cents as compared to approximately 5 cents when obtained from oranges available at the same season (Holmes, Patch, and Tripp, *New Engl. J. Med.* **228,** *8 (1943)*). Normally, however, tomatoes provide both a low-cost and a reliable source of the antiscorbutic vitamin together with other essential nutrients such as vitamin A and minerals.

Recent Nutrition Books

Handbook of Nutrition. A Symposium—Prepared Under the Auspices of the Council on Foods and Nutrition of the American Medical Association. Chicago. 1943. pp. 586.

THE NUTRITION FOUNDATION

The Nutrition Foundation was organized by food and related manufacturers to develop and support a comprehensive research and educational program in the science of nutrition. It functions chiefly through grants in support of research in university centers where there are strong graduate schools in the basic sciences. Nutrition problems of immediate importance in relation to the war emergency receive special consideration.

BOARD OF TRUSTEES

The Board of Trustees controls the corporation, determines its administration, establishes its policies, and in all ways manages its affairs in the public interest:

*Karl T. Compton, *Chairman*
*Cason J. Callaway, *Vice Chairman*
*Hugh O'Donnell, *Vice Chairman*

Joseph F. Abbott
James S. Adams
Frederick Beers
Carlyle H. Black
*Frank G. Boudreau
Frank E. Buck
Ellsworth Bunker
*O. C. Carmichael
*W. C. Coffey
Carle C. Conway
E. B. Cosgrove
Daniel W. Creeden
H. R. Dackett
William H. Duff, II
*Charles Wesley Dunn
J. Stafford Ellithorp, Jr.
Clarence Francis
Frank Gerber
Ralph Hayes
Henry J. Heinz, II
John Holmes
Adrian Joyce
*C. G. King
J. Preston Levis
C. R. Lindback
Charles P. McCormick
James McGowan, Jr.
John T. Menzies
Karl J. Monrad
Walter P. Paepcke
*Thomas Parran
Russell G. Partridge
Philip W. Pillsbury
George V. Robbins
Morris Sayre
Otto Schnering
*George A. Sloan
B. E. Snyder
R. Douglas Stuart
L. A. Van Bomel
L. A. Warren
*Ray Lyman Wilbur
*M. L. Wilson
*Stephen S. Wise

Asterisk (*) indicates representative of the public.

The quality and independence of the research program is safeguarded by the referee action and counsel of the Scientific Advisory Committee:

F. G. Boudreau
Milbank Memorial Fund
C. A. Elvehjem
University of Wisconsin
Icie M. Hoobler
Children's Fund of Michigan
P. E. Howe
Surgeon General's Office, U. S. Army
E. V. McCollum
The Johns Hopkins University
L. A. Maynard
Cornell University
J. R. Murlin
University of Rochester
R. C. Newton
Swift & Company
Lydia J. Roberts
University of Chicago
W. C. Rose
University of Illinois
W. H. Sebrell
National Institute of Health
H. C. Sherman
Columbia University
V. P. Sydenstricker
University of Georgia
F. F. Tisdall
University of Toronto
R. R. Williams
Bell Telephone Laboratories

Officers of The Nutrition Foundation

George A. Sloan, President
Charles Glen King, Scientific Director
Ole Salthe, Executive Secretary

CONTENTS

(Continued from Front Cover)

Biologic Value of Mixed Protein in Food 114

Vitamins and Physical Fitness 115

The Rice Problem 117

The Medical Appraisal of Calcium Nutrition 118

Corneal Vascularization in Vitamin Deficiencies 120

Thiamin and Riboflavin Enrichment 122

Destruction of Ascorbic Acid in the Gastrointestinal Tract 124

Pantothenic Acid Deficiency in Swine 125

Notes 127

The publication of NUTRITION REVIEWS has been undertaken to enable professionally trained people to keep abreast of current progress, and to have available an unbiased, authoritative review of the current research literature in the science of nutrition.

The Editorial Staff has been carefully selected so that the publication will merit in reasonable degree, the confidence of the medical profession and others who work directly with the public in the field of nutrition. The service provided by the publication is distinct from that of an abstract journal, a review for laymen, or an extensive scientific review. Since the reviews as originally prepared by a member of the Editorial Staff are frequently modified by special referees and may be further modified by the Editorial Office in preparing final copy for the press, the Editorial Committee feels that it is preferable not to affix the names of Editorial Staff members to individual reviews. There is thus a high degree of group responsibility on the part of the Editor and Assistant Editors.